

UNIVERSITY OF CALIFORNIA
COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
BERKELEY, CALIFORNIA

GRANULATION OF VALENCIA ORANGES

E. T. BARTHOLOMEW, WALTON B. SINCLAIR,
AND F. M. TURRELL

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GRANULATION OF VALENCIA ORANGES^{1, 2}

E. T. BARTHOLOMEW,³ WALTON B. SINCLAIR,⁴ AND F. M. TURRELL⁵

INTRODUCTION

GRANULATION MAY OCCUR in the Valencia orange, *Citrus sinensis* Osbeck, in all areas in California where this fruit is grown. Although it has been recognized in this state for many years, its economic importance was not fully realized until production reached the place where fruits of questionable quality had to give way to those of the best quality. At the present time, about 5 to 15 per cent (the percentage depending on the locality) of the Valencias picked in southern California from July to October cannot be shipped because of granulation.

In this country, granulation is found not only in California, but also in Arizona, Texas, and Florida. It is probably present in all countries where citrus is grown; for it has been reported and accurately described from Palestine, Egypt, India, Siam, South Africa, Australia, Brazil, Honduras, and the British West Indies. In California, granulation is confined almost entirely to Valencias, but it may occur to a slight extent in navel oranges from young trees in some areas. In other states in this country and in other countries, it may occur in these varieties and in almost any of the other commercial varieties of citrus, such as grapefruit, tangerines, and tangelos.

In general, no two citrus-growing areas use the same term to designate this disorder. For example, in Florida it is called "dry end," which is a good term in that little juice can be reamed from the stem-end half of a badly granulated fruit, but, strictly speaking, is erroneous because the affected juice sacs actually contain more water than the adjacent healthy ones. In Siam it is called "*Koa Sarn*," which means raw or uncooked rice. This term is applicable because in the advanced stages the affected juice sacs look somewhat like grains of rice. In Trinidad, British West Indies, it is referred to as "corkiness," but because of the physiological and histological condition of the affected sacs, this term does not appear so suitable.

In California the term "crystallization" has been used for many years and was probably originally suggested by the appearance of the little

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² Paper no. 422, University of California Citrus Experiment Station, Riverside, California.

³ Professor of Plant Physiology and Plant Physiologist in the Experiment Station.

⁴ Assistant Professor of Plant Physiology and Assistant Plant Physiologist in the Experiment Station.

⁵ Junior Plant Physiologist in the Experiment Station.

gas pockets which form in the juice sacs in the intermediate stages of development and which, to the unaided eye, may resemble crystals: there are no crystals in or on the affected juice sacs. "Granulation" appears to be a more descriptive term—one now widely used in California (1, 5).⁶ "Sclerocystosis"⁷ has been suggested as a technical term (2).

Unless otherwise stated, the Valencia variety of orange, *Citrus sinensis* Osbeck, is referred to in this paper in all cases where trees or fruits are mentioned.

DESCRIPTION OF GRANULATION AND DRY JUICE SAC

Granulation is not caused by a fungus, virus, or bacterium, but results from a series of abnormal physiological activities.

As a rule, granulation does not appear in the fruit in California to an appreciable extent until late in July, after the middle of the Valencia picking season. In 1932 a special study was made to determine how early in the development of the fruit granulation may be recognized. In a few cases it was found to be plainly evident in large fruits by the time they had reached the eight-to-one standard maturity test.⁸ Observations at earlier dates showed that the contents of some of the juice sacs in the stem end of large fruits from young trees had become gelatinized by the latter part of February, a month or more before the fruits normally matured in the locality from which they were obtained. Further observation and study have shown that gelatinization is a process through which all juice sacs must pass before reaching the condition known to the grower as typical granulation.

Some of the characteristics of granulation have already been described by Bartholomew, Sinclair, and Raby (2, 3). It is almost impossible to detect the disorder until the fruit has been cut. In the intermediate stages, however, a badly granulated fruit may feel harder, and in the last stage, softer, at the stem end than a healthy fruit. In the latter part of the season, the peel of a granulated fruit may turn a little greener, especially around the stem end, than a healthy fruit.

The preceding statements refer to fruit from the regular bloom. Each year a small percentage of off-bloom fruit is also produced. This fruit and the heavily shaded fruit from the regular bloom are usually not only off-color, but more subject to granulation than other fruit from the regular bloom. Because of the color differences, granulation is more easily detected, without cutting, in these fruits than in other fruits.

⁶ Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

⁷ "Sclero"=hard or tough, "cyst"=sac, and "osis"=state or condition.

⁸ The ratio of soluble solids to acids (8:1) required in the juice of oranges marketed in California.

Typical granulation begins in the stem end of the pulp and gradually progresses toward the styler end.⁹ A fruit is considered to be badly granulated when from one third to one half of the stem-end pulp has become affected. In some seasons and in some fruits, the extreme stem end of the pulp appears to be healthy, but small masses of slightly to moderately granulated juice sacs may be found near the "core," from one quarter to one third of the way in from either the stem or the styler end of the fruit, or from both ends.

Plate 1 (*A* and *B*) shows granulation in the stem end of the pulp of fruits that have been cut longitudinally and transversely. The peel in *B* of this figure appears to be excessively thick, but this is because the cut was made very near the stem end of the fruit. Plate 1, *I*, shows the cut surface of a longitudinal section of a healthy fruit.

Plate 1, at *C*, *G*, and *H*, shows a type of breakdown that is often referred to as "granulation." It may accompany granulation (plate 1, *H*) or appear independently (plate 1, *C*, *G*). The juice sacs do not at any time become enlarged or hardened as in granulation, but lose their water content and shrink until they become flattened or almost needle-shaped, their form depending upon the direction of lateral pressures. Isolated juice sacs of this kind are shown in plate 1, *E*, between healthy (plate 1, *D*) and granulated juice sacs (plate 1, *F*) from a fruit that was partially granulated. The condition first becomes evident in the longitudinal center of the pulp segment (plate 1, *G*) and in this again is different from granulation. Another characteristic of this disorder in which it is unlike granulation is its prevalence in intermediate- and small-sized fruits. It is often called "blossom-end granulation" because it is usually more pronounced in the styler end (and in the middle of the fruit) than in the stem end. Because of the specific characteristics of this condition of the pulp in Valencia and other citrus fruits, it seems desirable to distinguish it from granulation. "Dry juice sac," or simply "dry sac," is suggested at this time as being a good, descriptive, nontechnical term, and "xerocystosis,"¹⁰ as a technical term. During storage or transit, the apparent spread of dry juice sac in the fruit is greater than that of granulation. Like granulation, it is a physiological disorder.

FIELD EXPERIMENTS

In the course of field studies on granulation of Valencia oranges in California, from 1930 to 1938, inclusive, 125,600 fruits were cut and

⁹ The styler end of the citrus fruit is the end farthest from the point of attachment, often erroneously called the "blossom" end. The petals are attached at the base of the young fruit; therefore the blossom end and the stem end refer to the same portion of the fruit.

¹⁰ "Xero"=dry, "cyst"=sac, and "osis"=state or condition.

observed, and their condition was recorded. All of these fruits were cut and observed by the same person, so to this extent the personal element as a factor in judging the condition of the cut fruits was eliminated. The fruits came from approximately 1,500 trees in 65 different groves in Riverside, Orange, and Los Angeles counties. In addition, many thousand fruits from these and other groves have been used in making general observations and in biochemical, microchemical, and histological studies.

PRELIMINARY TESTS, 1930-1934

The results of some of the field experiments for 1930 to 1934, inclusive, have been previously described (2, 3), and at this time they will be given in summary form only.

The last of the 1930 Valencia crop at the Citrus Experiment Station, with the exception of the fruit of 10 thirteen-year-old trees, was picked during the last week in July. Many fruits from this pick were cut, but no granulation could be found. The fruits of the 10 trees that were left were picked and cut on different dates, as follows: on September 16 (3 trees), on October 10 (3 trees), and on November 13 (4 trees). On each of these dates the fruits from five different portions of each tree were segregated for cutting and observation. The average percentages of granulated fruits for each portion of all trees were: inside bottom, 71; outside north, 65; inside top, 56; outside east and west, 54; and outside south, 52. These results indicate that granulation is more prevalent in the fruit from some portions of the tree than from others. Other data obtained in this experiment indicate that fruit which is good in the earlier part of the season may become granulated if it is allowed to remain too long on the trees.

Trees in Cheesecloth Tents (1931).—The possibility that fogs or clouds, which decrease the amount of available sunshine, might be a factor in causing granulation suggested the following tests. Five fourteen-year-old Valencia trees at the Citrus Experiment Station were enclosed within a single large tent made of thin cheesecloth. The direct sunshine did not strike the foliage, but the tent was well ventilated by means of louver openings. In a near-by grove of six-year-old Valencias, each of 6 trees was enclosed singly in a tent of the same material. Three of the tents were entirely closed, and there was no ventilation except through the cheesecloth. The other 3 tents were especially well ventilated, for there were no north or south sides, and the tops extended only far enough to prevent the direct rays of the sun from striking the foliage. All tents were installed in May and removed in October. In each case, the trees adjacent to the tents served as controls.

Test cuts of the fruits from these trees were made on September 10 and

October 20 and included a total of 8,993 fruits. Every mature fruit on each tree was cut and observed. The trees were found to have produced average amounts of granulation as follows: 5 trees in large tent and 5 adjacent controls, 17 and 35 per cent, respectively; 3 trees in small closed tents, 3 in small open tents, and 6 adjacent controls, 57, 57, and 58 per cent, respectively. The shading of the trees within the large tent evidently did not cause an increase in the amount of granulation. In fact, the results indicate that the shading may have caused a reduction in granulation. This may be explained, however, on the basis that these trees were naturally low producers of granulation (see "Tree Differences and Constancies in the Production of Granulation," p. 12). The tents on the small trees apparently had no effect on the amount of granulation. The results show definitely that, under the conditions of this test, the reduction in the amount of sunshine available for the trees did not cause an increase in the percentage or severity of granulation in the fruit.

Excessive Growth of Fruits at Stem End and Granulation.—Early in the study of granulation, the opinion was expressed by some growers that a secondary growth occurred in the stem end of at least some of the Valencia fruits during the summer and that this secondary growth was caused by, or was the cause of, granulation. Information concerning this possibility was sought in 1931 by volumetrically measuring the comparative increase in size that occurred in the stem and styler halves of fruits during the period from early summer to early fall. The equator of each fruit was marked by an inked thread fastened to a specially devised spring wire. The volume of the stem half and of the styler half of each fruit was then determined by the amount of water displaced by each half in another specially devised piece of apparatus. Similar volumetric measurements were made on the same fruits at the time they were to be cut and examined for granulation. Ten of the largest fruits on each of 10 thirteen-year-old trees and on each of 5 six-year-old trees were measured.

The results of these tests were not entirely conclusive. The fruits from the thirteen-year-old trees showed a slightly greater increase in the stem halves of the good fruits than in those of the granulated fruits, but the difference is probably not significant. The average volume increase of the stem half was 3 cc greater than that of the styler half for the good fruits and only 2 cc greater for the granulated. With the six-year-old trees, the differences were greater: the average volume increase of the stem half was 10 cc greater than that of the styler half for the good fruits and 14 cc for the granulated—a reversal of the results shown by the good and the granulated fruits from the thirteen-year-old trees.

The results of these tests at least indicate that there is no definite rela-

tion between granulation and enlargement of the fruit at the stem end. No numerical data have been obtained, but continued observation over the period from 1931 to 1938 has led to the conclusion that the more nearly spherical fruits are no less likely to be granulated than those which are more elongated. As a rule, in the coastal areas where granulation is more abundant, the fruits are more spherical than they are in the interior districts where granulation is less abundant.

The data were next examined to determine whether there was any more granulation in the fruits that had made the greatest increase in size, as a whole, than in the other fruits. The average volume increase of all the good fruits from the older trees was 7.6 per cent, while that of the granulated fruits was 8.5 per cent. When only 10 of the largest fruits in each of these two groups were considered, the results were just the reverse: the 10 good fruits showed an increase of 14.9 per cent, while the 10 granulated fruits had increased only 12.5 per cent. The fruits from the young trees did not show this reversal. The average volume increase of all the good fruits from the young trees was 14.9 per cent, while that of the granulated fruits was 19.6 per cent. When only 10 of the largest fruits in each of these two groups were considered, it was found that the 10 good fruits had increased 17.7 per cent, while the 10 granulated fruits had increased 28.3 per cent.

The lack of consistent results in these tests on the relation between size increase of fruits and granulation is an example of the inconsistencies encountered all through the course of study of granulation.

The Effect of Sprays (1931-1934).—In the summer of 1931, 3 thirteen-year-old trees at the Citrus Experiment Station were sprayed with lime. In the fall, the fruits of these trees were cut and observed, and very little granulation was found.

The lime-spray test was repeated in 1932 on a much larger scale in a fifteen-year-old grove at the Citrus Experiment Station and in a ten-year-old grove near Santa Ana. The latter grove was chosen because in previous years it had produced a great deal of granulation. Thirty trees in each grove were sprayed with a lime whitewash composed of 40 pounds of slaked lime and 1 pound of Kayso spreader in each 100 gallons of water. Twenty of the trees in each grove were sprayed in March. In May, 10 of these trees were resprayed, and 10 more were given a first application. Three test cuttings of the fruit from these trees and 10 control trees were made during the late summer and early fall. In each test, 50 fruits were chosen at random from both the north and the south half of each tree. This called for the cutting of 24,000 fruits. From two to four transverse cuts were made on each fruit to determine the absence or presence, and the severity, of granulation. Since no significant differ-

ences resulted from the different methods of spraying, the results of all of them are combined for comparison with the unsprayed trees.

The consistency of the results of the random sampling is indicated in figure 1. The granulation percentages for 100 fruits from each of 10 trees were determined, and then at once a second, similar determination was made on other fruits from the same group of 10 trees. The figure shows that there was very close agreement in the two tests.



Fig. 1.—Comparative results of two successive granulation tests made on the same day. In each test, 100 fruits were picked at random from each of the same 10 trees (nos. 14–23), and percentages of granulated fruits were determined. (The vertically hatched columns represent the results of the first pick.)

In order to determine the comparative numbers of fruits of different sizes and the degree of granulation, a field sizing and grading board was devised and constructed (plate 2, A). To prevent unnecessary complication in compiling the results, the fruits were graded into only five sizes: 100's, 150's, 200's, 288's, and 344's. Each of these was arbitrarily chosen to represent a different group within the range of sizes (80's to 360's) used in the standard pack of the California Fruit Growers' Exchange.

The sizing and grading board was so constructed that as a fruit was picked and sized, then cut and inspected, the moving of an indicator on the proper dial recorded its size and also its quality, such as "good," or, with reference to granulation, "slight," "moderate," or "bad."

Unfortunately for the experiment, the fruit from the trees in the grove at the Citrus Experiment Station did not have enough granulation in it to warrant making the complete test. The cutting of 3,100 fruits from these trees, at intervals during the latter part of the picking season, showed a maximum of only 3 per cent granulation on any one tree, and in these fruits the granulation was very slight.

Granulation in the grove of ten-year-old trees near Santa Ana was bad and afforded an excellent opportunity for determining the effects of the lime spray. The results of this test are shown in summary form in table 1. These data show that 67 per cent of the fruit from the unsprayed trees and only 34 per cent of that from the sprayed trees was granulated. The data also show that fruit from the sprayed trees was much less severely affected than that from unsprayed trees. (Even fruits containing only two or three affected juice sacs were classed as granulated.)

TABLE 1
GRANULATION IN FRUITS FROM UNSPRAYED AND LIME-SPRAYED TREES,
SANTA ANA, 1932*

Treatment	Proportion of fruits granulated in various sizes						Severity of granulation, all sizes		
	100's	150's	200's	288's	344's	All sizes	Slight	Moder- ate	Bad
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Unsprayed.....	95	84	62	29	10	67	18	34	15
Lime-sprayed.....	80	70	27	8	1	34	19	7	8

* Samples consisted of 50 fruits, chosen at random, from both the north and the south half of each tree—10 trees unsprayed and 30 sprayed. Fruits were segregated into the five sizes in a field sizing board and were cut at once for observation.

An attempt was made in 1933 to determine whether the reduction in the amount of granulation in the fruit from the lime-sprayed trees was caused by a chemical or by a physical effect of the lime. The tests were made in the same 2 groves in which the 1932 tests had been made. Ten trees in one row in each grove were sprayed with an aqueous mixture of barium sulfate and cornstarch (75 pounds barium sulfate, 38 pounds uncooked cornstarch, and 1.9 pounds of agar, in 150 gallons of water). Ten other trees in a second row in each grove were sprayed with a mixture composed of 6.3 pounds of calcium nitrate and about 1 pound of blood albumin in 150 gallons of water. The amount of calcium nitrate used was calculated to give about 10 ounces to each tree. In a third row in each grove the 10 trees were not sprayed, but 115 to 120 of the largest fruits on each tree were dipped in a lime whitewash of the same concentration as had been used as a spray in 1932. In a fourth row in the grove at the Citrus Experiment Station, a similar number of fruits were enclosed in waterproof bags, and the trees were then sprayed with the lime whitewash. The bags were removed as soon as the foliage had dried. In each grove, the fruit on an adjacent block of unsprayed trees served as controls for the spraying tests. Fruits borne adjacently, on the same trees, served as controls for the dipped and bagged fruits. The spray and other applications were made during the first week in May.

Again, as in 1932, preliminary tests made at monthly intervals showed that there was not enough granulation in the Citrus Experiment Station grove to warrant the completion of the test. While this was discouraging so far as the test was concerned, it substantiated the observation that granulation is more prevalent in coastal than in interior districts.

In the grove near Santa Ana, two granulation cutting tests were made, one in early July and the other 6 weeks later. In the two tests, 11,000 fruits were cut and examined. The total percentages of granulated fruits from the trees which received the different spray treatments were: sprayed with barium sulfate and cornstarch, 69; with calcium nitrate, 72; unsprayed (controls), 87. Neither the barium-starch nor the calcium nitrate sprays had any very great controlling effect on granulation, either as to amount or severity. It should be stated that the barium-starch mixture did not make the trees nearly so white as the lime spray used in 1932.

The fruits dipped in limewater showed 63 per cent, and the controls from the same trees, only 59 per cent, granulation. It would appear from these results that the dipping in lime had caused a slight increase in the amount of granulation. Such was probably not the case, because the records show that 50 per cent of the dipped fruits and only 28 per cent of the controls were size 150 or larger. The large fruits are more likely to granulate than the small ones. As already stated, 115 to 120 of the *largest* fruits on each tree were dipped, so that the fruits which were cut from the same trees for controls were necessarily smaller in size. The trees bearing the dipped fruits and their controls were in a portion of the grove which normally produced a comparatively low percentage of granulation. This probably explains why a lower percentage of these fruits than of those in the spray plots were granulated.

In 1934 the lime-spray tests were repeated in the grove near Santa Ana. Plots of 20 trees each were sprayed with 10, 15, and 20 pounds of lime and 1 pound of Kayso spreader to each 100 gallons of water. Twenty adjacent trees—one row of 10 trees on the north side and another on the south side of the plot—served as controls. The spray was applied on April 11. Because of the lack of granulation in the two previous years, the test was not made in the grove at the Citrus Experiment Station.

As in previous years, 50 fruits were picked at random from both the north and the south half of each tree for cutting—a total of 8,000 fruits. This year only one fruit-cutting test was made. The results were not conclusive. Granulated fruits on the trees to which the 10, 15, and 20 pounds of lime were applied averaged 62, 51, and 40 per cent, respectively, but the control trees produced only 48 per cent. Probably the

factor of chance operated here as, apparently, in the large tent experiment in 1931. While the number of fruits affected was about the same for this year (1934), the degree of severity of granulation in the fruits was less than it had been in the two previous years, not only in this grove, but in all Valencia-growing districts in California.

TREE DIFFERENCES AND CONSTANCIES IN THE PRODUCTION OF GRANULATION

In the course of these experimental tests, from 1930 to 1938, it was found that some trees produce a larger number of granulated fruits and fruits more severely granulated than adjacent trees in the same grove. The granulation yields for the years 1933 to 1937, on the 4 trees on which this difference was first especially noticed, are recorded in table 2. One

TABLE 2

TREE DIFFERENCES AND CONSTANCIES IN THE PRODUCTION OF GRANULATION,
1933-1937*

Tree no.	Proportion of fruits granulated					
	1933	1934	1935	1936	1937	5-year average
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
15.....	89	63	75	41	43	62
16.....	96	78	71	60	59	73
22.....	32	40	9	16	2	20
25.....	39	35	6	5	7	18

* Trees located near Santa Ana and not treated. Fruits were selected as for data in table 1, except in 1937, when all fruits on one large limb on each tree were cut (trees had been top-worked and all other limbs removed).

hundred fruits per tree were used in making these determinations, except in 1937, as explained in the following paragraph.

The first 2 trees listed in the table show a much higher percentage of fruits affected than the other 2 trees, and records not given in the table show that the severity of granulation in the fruits from these trees was much greater than in fruits from the other 2 trees. In 1935, for trees 15 and 16, and in 1933 and 1937, for trees 22 and 25, the percentages were reversed from their relative positions in other years. It should be stated that the percentages for 1937 were obtained from fruits from only one large limb on each tree. All the other large limbs had been removed from the trees when they were top-worked in the preceding summer (see p. 17). On the single limbs of trees 15, 16, 22, and 25, there were 73, 96, 135, and 298 fruits, respectively; all fruits were picked and cut.

In order to get a more complete record of possible differences in

amounts of granulation produced by different trees in the same grove, fruit tests were made in 27 groves in Orange and Los Angeles counties in 1936. Five trees near each corner of each grove (20 trees in each grove) were selected and tagged. The fruit was allowed to remain on these trees until late in the picking season to give the fruit a good chance to granulate. The trees in the different groves ranged from eight to thirty years of age. When the test cuttings were made, 25 of the largest fruits

TABLE 3

GROVE AVERAGES AND TREE DIFFERENCES IN GRANULATION PRODUCTION, 1936*

Grove no.	Age of trees	Fruits granulated		Grove no.	Age of trees	Fruits granulated	
		Grove average	Range in individual trees			Grove average	Range in individual trees
	<i>years</i>	<i>per cent</i>	<i>per cent</i>		<i>years</i>	<i>per cent</i>	<i>per cent</i>
1.....	8	91	76- 96	15.....	22	43	0-100
2.....	20	35	4- 60	16.....	12	35	12- 64
3.....	25	45	12- 76	17.....	22	23	0- 84
4.....	9	51	16- 72	18.....	19	25	0- 56
5.....	9	38	4- 56	19.....	19	25	0- 84
6.....	20	29	4- 76	20.....	19	16	0- 56
7.....	30	14	0- 28	21.....	19	24	0- 72
8.....	16	37	0- 84	22.....	12	5	0- 52
9.....	25	23	0- 78	23.....	22	20	0- 48
10.....	12	60	12-100	24.....	?	34	0- 84
11.....	12	44	12- 96	25.....	15	27	0- 68
12.....	12	14	0- 48	26.....	18	5	0- 28
13.....	10	31	8- 68	27.....	18	17	0- 40
14.....	25	18	0- 48				

* Tests included 20 trees in each of 27 groves located in Orange and Los Angeles counties, a total of 540 trees. Twenty-five of the largest fruits from each tree, a total of 13,500 fruits, were tested.

were chosen from each tree, making a total of 13,500 fruits. The largest fruits were chosen because they were the ones that were most likely to be granulated.

The results of these 1936 tests are presented in table 3, which shows the minimum and maximum percentages of granulation on trees within a given grove and the average percentage for each grove.

Similar fruit cuttings were made in 14 of the 27 groves over a period of three successive years (1936-1938) to obtain further data on tree constancy in the production of granulation. These tests entailed the cutting of 15,000 additional fruits during 1937 and 1938. The data on only 4 of these groves are given (table 4), but they are typical of results obtained in all of them. About half of the trees were constant over the entire three-year period in the production of either large, moderate, or small numbers of granulated fruits, while many of the trees were constant in only two of the three years.

TABLE 4
TREE TENDENCIES TOWARD CONSTANCY IN THE PRODUCTION OF GRANULATION,
1936-1938

Row and tree no.	Number of fruits granulated (of 25 fruits cut)			Row and tree no.	Number of fruits granulated (of 25 fruits cut)		
	1936	1937	1938		1936	1937	1938
Grove no. 1				Grove no. 5			
Row 4:				Row 3:			
Tree 4.....	23	22	24	Tree 1.....	14	16	19
Tree 5.....	22	25	25	Tree 2.....	11	11	4
Tree 6.....	24	20	25	Tree 3.....	15	19	15
Tree 7.....	23	17	20	Tree 4.....	7	20	14
Tree 8.....	25	24	25	Tree 5.....	7	17	15
Row 7:				Row 5:			
Tree 5.....	23	25	25	Tree 6.....	5	10	5
Tree 6.....	23	22	25	Tree 7.....	6	18	7
Tree 7.....	20	23	23	Tree 8.....	12	12	11
Tree 8.....	24	23	24	Tree 9.....	11	14	10
Tree 9.....	23	23	24	Tree 10.....	7	22	8
Row 15:				Row 11:			
Tree 6.....	23	20	23	Tree 6.....	9	9	10
Tree 7.....	19	13	19	Tree 7.....	2	6	7
Tree 8.....	22	13	22	Tree 8.....	1	9	15
Tree 9.....	22	11	25	Tree 9.....	10	6	4
Tree 10.....	24	19	24	Tree 10.....	5	13	9
Row 19:				Row 14:			
Tree 3.....	24	23	24	Tree 1.....	24	24	22
Tree 4.....	24	19	23	Tree 2.....	9	18	10
Tree 5.....	22	24	22	Tree 3.....	10	12	11
Tree 6.....	20	17	15	Tree 4.....	13	10	7
Tree 7.....	23	10	18	Tree 5.....	14	15	13
Grove no. 3				Grove no. 9			
Row 5:				Row 3:			
Tree 3.....	11	10	13	Tree 34.....	9	8	19
Tree 4.....	16	3	22	Tree 35.....	0	2	5
Tree 5.....	7	12	14	Tree 36.....	3	3	5
Tree 6.....	11	14	7	Tree 37.....	10	1	8
Tree 7.....	7	25	18	Tree 38.....	12	3	16
Row 4:				Row 4:			
Tree 14.....	3	5	10	Tree 51.....	2	13	6
Tree 15.....	8	9	9	Tree 52.....	3	0	24
Tree 16.....	15	15	4	Tree 53.....	6	6	6
Tree 17.....	18	19	14	Tree 54.....	3	1	3
Tree 18.....	19	10	18	Tree 55.....	7	7	4
Row 11:				Row 7:			
Tree 18.....	15	17	18	Tree 49.....	4	2	3
Tree 19.....	8	3	5	Tree 51.....	19	13	13
Tree 20.....	12	12	9	Tree 52.....	0	2	4
Tree 21.....	9	17	0	Tree 53.....	5	2	2
Tree 22.....	16	24	17	Tree 54.....	4	2	8
Row 10:				Row 8:			
Tree 4.....	—*	4	7	Tree 35.....	1	5	15
Tree 5.....	13	5	—*	Tree 36.....	6	—*	19
Tree 7.....	8	5	4	Tree 37.....	2	—*	10
Tree 8.....	12	13	19	Tree 38.....	10	—*	8
Tree 9.....	6	8	0	Tree 39.....	8	—*	5

* Data not available; these trees had been picked by mistake by the pickers.

The fact that certain trees in a given grove produce more granulation than the adjacent trees in the same grove is shown in histogram form in figure 2. The figure also shows that some trees are comparatively con-

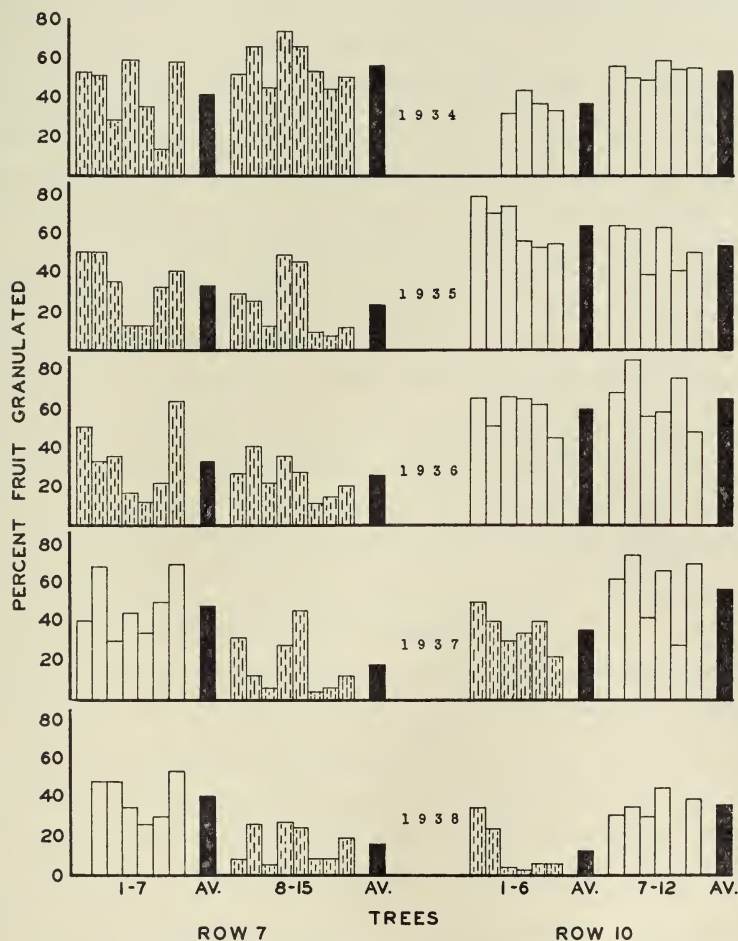


Fig. 2.—Differences and constancies of adjacent trees in the production of granulation over a period of five years. The vertically hatched columns represent the infrequently irrigated trees and show the reduction in granulation effected by reduced amounts of irrigation water. Trees 1 and 2, row 10, were not included in the 1934 test; and the fruit from tree 1, row 7, and from tree 11, row 10, was not included in the 1938 data because the former tree had been partially picked by mistake, and the latter had been killed by gophers.

stant in the production of granulation, and others are not. Figure 2 also illustrates the reduction in granulation effected by reduced amounts of irrigation water. (For results of these tests, see "Reduction in Frequency of Application and in Amounts of Irrigation Water," p. 18.)

EXPERIMENTS IN BUDDING AND TOP-WORKING

When it was shown that certain trees within a given grove exhibited at least a fairly constant tendency to produce much or little granulation from year to year, it seemed advisable to determine whether this tendency might be transmitted through the bud. Forty buds were, accordingly, taken from each of the 4 trees mentioned in table 2, 2 of which (trees 15 and 16) had produced much granulation and 2 of which (trees 22 and 25) had produced little. Twenty of the 40 buds from each tree were inserted in sweet-orange seedlings, and the remainder in sour-orange seedlings. The seedlings were budded in April, 1934.

In May, 1935, 100 of the best of the 160 young budded trees were removed from the nursery and planted at the Citrus Experiment Station. Approximately equal numbers of the best progenies of each of the 4 trees were selected, and of those selected, half were on sweet-orange and half were on sour-orange stock.

The bud-transmission tests were extended in 1935 by taking 40 buds from each of 2 Valencia trees at the Citrus Experiment Station and growing them on sweet- and sour-orange stocks, as in the preceding test. One year later, 40 of the best of the young budded trees were selected as before and planted in the same grove, adjacent to the plantings of 1935. Of the 2 trees from which the buds were taken, 1 had produced granulation from year to year in such abundance that most of the fruit was almost worthless a few weeks after it had reached the standard soluble solids-to-acid ratio. The fruit could remain on the other tree until late summer and still show very little granulation.

It is too early yet to say what the outcome of these bud-transmission tests will be. But the first fruit-cutting tests, which were made in September, 1938, showed the following results: Of the 100 trees planted in 1935, 22 bore 79 fruits; 21 of these 22 trees were on sour-orange root-stock; and most of the fruits were small, 72 per cent of them being size 200 or smaller. Seventeen of the 79 fruits were granulated, and of these, 13 came from the trees started from buds taken from the 2 trees (table 2, trees 15 and 16) which had produced much granulation. None of the 40 trees planted in 1936 bore fruit. In 1939 the fruits were small and a comparatively small percentage of them were granulated. The tests for this year were of special interest, however, because, in general, the results were the reverse of those of the preceding year. The number of granulated fruits on the young trees started from buds from trees 15 and 16 (high producers of granulation) was slightly lower than on the young trees from buds from trees 22 and 25 (low producers of granulation): 24 and 26 per cent, respectively. Definite information on bud

transmission of granulation cannot be obtained from these trees for at least ten years from the time of planting.

In 1936 the 4 trees mentioned in table 2 were cross-top-worked, as a further check on the possibility that granulation tendencies may be transmitted through the bud. Buds from trees 15 and 16, which had produced much granulation, and from the good tree at the Citrus Experiment Station, which had produced little, were inserted in trees 22 and 25, which had produced little granulation. The second step was just the reverse: buds from trees 22 and 25 and from the tree at the Citrus Experiment Station were inserted in trees 15 and 16. It is too early yet to determine what the results of these tests will be.

With reference to the possible effect of parentage on the production of granulation, Mr. Lombard¹¹ states that 2 orchards started from buds from the same parent tree (no. 2-18-20), but planted on different types of soil, produced 57 and 54 per cent granulation, respectively. Two other orchards started from buds from a different parent tree (no. 4-15-30) produced only 10 and 8 per cent granulation. The progenies from both parent trees were on the same kind of rootstock. From these and similar results, Mr. Lombard concludes that "It is probably advisable to select parent trees whose progenies, say up to ten years old, are relatively free of crystallization [granulation]."

GRANULATION AND SCALY BARK

Some growers are of the opinion that scaly bark (psorosis) may be a factor in the production of granulation. During the study of granulation, this factor has been given considerable attention. In several of the groves where granulation studies were made, there was no visible sign of scaly bark, at least not on the trunks and limbs. In other groves, the trees were badly affected on either trunk or limbs, or on both, and some of the trees were so badly affected that the foliage was sparse and unhealthy in appearance.

In 1934 a special study was made on a block of 80 trees near the center of a grove that was noted for its production of large amounts of granulation. The observations on the presence or absence of scaly bark on these trees were made at a time when the trees bore a flush of new growth, for the leaf symptoms can be most easily detected when the leaves are young. H. S. Fawcett,¹² who is the best-known authority on this disease, assisted in making the observations. In determining the percentages of

¹¹ Lombard, T. H., Assistant Manager, Rancho Sespe, Fillmore, California, in a letter to the senior author dated July 21, 1939.

¹² Fawcett, H. S., Professor of Plant Pathology and Plant Pathologist in the Experiment Station.

granulation on these trees, 50 fruits from both the north and the south half of each tree were picked and cut.

The observations on scaly bark showed that of the 80 trees, 6 had lesions on both trunk and limbs, 7 had lesions on the trunk only, and 10 had lesions on the limbs only. Every one of the 23 trees that bore scaly-bark lesions on the trunk or limbs also showed the disease in the leaves in virus form. In addition to these, there were 9 other trees that showed the disease as a leaf symptom only; there were no lesions on either trunk or limbs. Foliage production did not appear to be reduced even on the trees having lesions on the trunk and limbs.

At least so far as these 80 trees were concerned, there appeared to be no relation between presence or absence of scaly bark and the prevalence of granulation. Of the 12 trees that produced the greatest number of granulated fruits, none showed scaly-bark lesions on the trunk or limbs, and only 3 showed it in the leaves in virus form.

The specific tests for determining the possible relation between the presence of scaly bark and the production of granulation were conducted over a period of only the one season, and the results may be open to criticism for that reason. The general observations made between 1934 and 1938, however, tend to substantiate the findings of the specific test in 1934. Occasional trees were found that were badly affected with scaly bark and produced more than the average number of granulated fruits, but there were many trees similarly affected that produced less than the average number. From the tests and observations cited, it seems to be safe to say that there is no direct relation between the presence of scaly bark and the production of granulation, at least until there is an evident reduction in foliage; even then the relation may be questioned.

REDUCTION IN FREQUENCY OF APPLICATION AND IN AMOUNTS OF IRRIGATION WATER

In 1934, tests were begun in a grove near Santa Ana to determine the possible effect of less frequent applications and of reduced amounts of irrigation water on the prevalence of granulation in the fruit.¹³ The experiment ran from 1934 through 1938. The trees were twelve years old when the experiment began. The test plots were small, including only 27 trees in all, but the manner in which the experiment was conducted was such that the results appear to be conclusive and reliable for this type of soil and for this locality.

¹³ From May, 1934, to September, 1936, the irrigations in this experiment were made by the grove owner under the direction of H. E. Wahlberg, Farm Advisor of Orange County. From September, 1936, until the experiment was terminated in 1938, soil determinations, water measurements and irrigations, and fruit measurements were made by M. R. Huberty and his assistants, of the University of California Division of Irrigation.

Soil Characteristics and Sampling.—The trees were growing on soil classed as Yolo loam, interspersed in places, however, with layers of heavier soil, almost clay. The texture (as indicated by moisture equivalent) and other characteristics of the soil in each of the plots on June 28, 1937, are shown in table 5.

Between July 20, 1936, and August 16, 1938, when the tests were terminated, soil samples were taken 34 times on each of the four plots to

TABLE 5
CHARACTERISTICS OF SOIL* IN IRRIGATION PLOTS

Plot no.	Foot depth	Permanent wilting point†	Moisture equivalent‡	Apparent specific gravity
		<i>per cent</i>	<i>per cent</i>	
3‡	{ First.....	5.5	15.7	1.4
	{ Second.....	5.1	15.0	1.2
	{ Third.....	10.4	26.3	1.0
	{ Fourth.....	8.5	21.7	1.2
4§	{ First.....	6.1	16.8	1.4
	{ Second.....	5.8	15.5	1.1
	{ Third.....	11.0	27.0	1.1
	{ Fourth.....	9.5	22.8	1.2
5§	{ First.....	4.9	13.1	1.5
	{ Second.....	7.0	20.6	1.2
	{ Third.....	11.0	26.0	1.2
	{ Fourth.....	8.1	20.2	1.3
6‡	{ First.....	5.3	14.9	1.5
	{ Second.....	6.1	15.9	1.1
	{ Third.....	11.3	26.5	—
	{ Fourth.....	11.2	24.8	1.2

* Yolo loam, interspersed with layers of heavier soil.

† Amount of water at this point as a percentage of the oven-dry weight of the soil.

‡ "Wet" plot, September, 1936, to August, 1938.

§ "Dry" plot, September, 1936, to August, 1938. (For explanation of "wet" and "dry" plots, see footnote 14, p. 20.)

determine soil-moisture content. Five samples were taken from each of two definite areas in each plot at each sampling. The two sampling areas had been staked off in advance in each plot. One area ran diagonally across the row from center to center; the other ran diagonally across the center from near the skirt of one tree to near the skirt of the next tree in the adjoining row. Samples were taken at 1-foot levels to a depth of 4 feet.

Irrigation Treatment.—From May, 1934, to September, 1936, the water was applied to both test and control rows at the time of the regular irrigations for that grove; but the test row (plot 1) and adjacent row on each side received water on alternate sides only at each irrigation. In this way these three rows received only approximately half as much

water as the control row (plot 2) and adjoining rows, which were watered on both sides in the usual manner at each irrigation. The furrow method of irrigation was used.

From September, 1936, to August, 1938, the test and control rows and their respective two adjacent rows, were divided into two plots each, as follows: test row (plot 1, trees 1-15) into plot 3 (trees 1-7) and plot 4 (trees 8-15); control row (plot 2, trees 1-12) into plot 5 (trees 1-6) and plot 6 (trees 7-12). Adjacent rows, one on each side, were included to make sure that the entire root systems of the trees in the test and control rows should be subjected to their respective treatments.

Plots 3 and 6 were watered when the moisture content of the soil in the first foot had nearly reached the wilting point. Plots 4 and 5 were not watered until the moisture content of the soil in the fourth foot was approaching the wilting point. Thus, from September, 1936, to August, 1938, plots 4 and 5 were alternately wet and dry, except when rain fell, and plots 3 and 6 were wet practically all the time.¹⁴

The exact amounts of water given to plots 1 and 2 are not known. After these plots had been divided into the four new plots (plots 3, 4, 5, and 6), however, the basin method of irrigation was used, and a water meter was employed so that definite and known amounts of water could be applied as desired. In 1937, plots 3 and 6 (wet) received four applications, totaling 8.3 inches. Plots 4 and 5 (dry) received only one application, 2.3 inches. In 1938, up to August 5, when the experiment was terminated, plots 3 and 6 received a total of 6.3 inches in two applications; and plots 4 and 5, one application of 3.6 inches. These applications were sufficient to keep the moisture content above the wilting point in the first foot in the wet plots and in the fourth foot in the dry plots. It should be stated, however, that the trees in the dry plots showed some wilting just before irrigation when the temperature was high and the relative humidity low.

Granulation Tests.—The results of the granulation tests on the plots which received differential water treatments are shown in table 6. Fruits were cut and observed each year from 1934 to 1938, inclusive. Fifty fruits were picked at random from both the north and the south half of each tree, except in 1937, when only 25 of the largest fruits were taken from each half.

¹⁴ In the succeeding description and discussion of this irrigation experiment and in table 6, plot 1, which was irrigated on alternate sides of the rows at each irrigation, and plots 4 and 5, which were not irrigated until the moisture in the fourth foot had almost reached the wilting point, will be referred to as "dry" plots; plot 2, irrigated in the usual manner, and plots 3 and 6, irrigated when the moisture in the first foot had almost reached the wilting point, will be called "wet" plots. The amounts of water applied to these plots are expressed in acre-inches per acre, or average depth inches.

As already mentioned, the two original plots (1 and 2) were not divided into four plots until 1936, but the division (plots 1A, 1B, 2A, 2B) is shown in the table for the entire period, so that the behavior of the trees before the division was made could be followed. Although the plots

TABLE 6
PERCENTAGES OF GRANULATED FRUITS FROM TREES ON WET AND DRY PLOTS,
1934-1938*

Row and tree no.	July 31, 1934	July 16, 1935	Sept. 1, 1936	Aug. 31, 1937	Aug. 31, 1938	Row and tree no.	July 31, 1934	July 16, 1935	Sept. 1, 1936	Aug. 31, 1937	Aug. 31, 1938
Row 7	Dry treatment (plot 1A†)			Wet treatment (plot 3)		Row 10	Wet treatment (plot 2A†)			Dry treatment (plot 5)	
	per cent	per cent	per cent	per cent	per cent		per cent	per cent	per cent	per cent	per cent
Tree 1.....	53	50	50	40	—	Tree 1.....	—	79	65	50	35
Tree 2.....	51	50	32	68	48	Tree 2.....	—	70	51	40	24
Tree 3.....	28	35	25	30	48	Tree 3.....	32	74	66	30	4
Tree 4.....	59	12	16	44	35	Tree 4.....	44	56	65	34	3
Tree 5.....	35	12	11	34	26	Tree 5.....	37	53	62	40	6
Tree 6.....	13	32	21	50	30	Tree 6.....	33	54	45	22	6
Tree 7.....	58	40	63	70	53						
Average.....	42	33	31	48	40	Average.....	37	64	59	36	13
Row 7, cont.	Dry treatment (plot 1B†)			Dry treatment (plot 4)		Row 10, cont.	Wet treatment (plot 2B†)			Wet treatment (plot 6)	
	per cent	per cent	per cent	per cent	per cent		per cent	per cent	per cent	per cent	per cent
Tree 8.....	52	29	26	32	8	Tree 7.....	56	64	68	62	31
Tree 9.....	66	25	40	12	26	Tree 8.....	50	62	85	74	35
Tree 10.....	45	12	21	6	5	Tree 9.....	49	39	56	42	30
Tree 11.....	74	49	35	28	27	Tree 10.....	59	63	58	66	45
Tree 12.....	66	45	37	46	24	Tree 11.....	54	41	76	28	—
Tree 13.....	53	9	11	4	8	Tree 12.....	55	50	48	70	39
Tree 14.....	44	7	14	6	8						
Tree 15.....	50	11	20	12	19						
Average.....	56	23	26	18	16	Average.....	54	53	65	57	36

* Samples consisted of 50 fruits chosen at random from both the north and the south half of each tree, except in 1937, when 25 of the largest fruits were similarly chosen. Fruits were picked and cut on the dates given.

† Plots 1A and 1B were given the same treatment for the three years 1934-1936, but are averaged separately because plot 1A became plot 3 in 1937 and plot 1B became plot 4 then. A similar situation exists in plots 2A and 2B.

were small, the results show that there was a direct reduction in the amounts of granulation on the plots that received the reduced amounts of irrigation water. The differential applications of water in 1934 were begun too late to have an effect on the prevalence of granulation that year; therefore the amounts on the trees in each plot were very much the same. In the succeeding years the difference between the amounts of granulation on the wet and dry plots was pronounced.

In 1935 and 1936, trees 1 to 15, plot 1 (dry), produced averages of 28 and 29 per cent, respectively, of granulated fruits; while averages for trees 1 to 12, plot 2 (wet), were 59 and 62 per cent, respectively.

The results for 1937 and 1938 were even more striking and convincing. During these two years, plot 3 (wet) produced appreciably more granulation than it had in the two preceding years when it had served as a portion of the original plot 1 (dry) (plot 1A in table 6); plot 6 (wet), which was frequently irrigated over the entire period (as plot 2B in 1934–1936), continued to produce a comparatively large amount of granulation; plot 4 (dry), which was infrequently irrigated over the entire period (as plot 1B in 1934–1936), continued to produce small amounts of granulation; plot 5 (dry) produced much granulation in 1935 and 1936 while it was serving as a portion of plot 2 (wet) (plot 2A in table 6), but the amounts were very much smaller in 1937 and 1938 when it served as a dry plot. The fruits were small on all four plots in 1938, and the amounts of granulation were correspondingly small.^{14a}

The reduction in the amount of water applied affected some trees more than others. For example, from 1935 to 1938 the reduction in amounts of granulation on trees 13, 14, and 15, of row 7, was considerably greater than on trees 11 and 12 of the same row (table 6 and fig. 3). Other comparable cases may be found in the table. Exceptions similar to these have been noted in discussing some of the preceding tests.

Fruit Sizes and Yields.—In order to determine whether there might be any difference in the growth rates and sizes of the fruits on the wet and dry plots, 15 fruits on each of 2 trees in each of plots 3 to 6 were tagged and measured. Fruit size was determined by measuring the equatorial circumference in millimeters; from this figure, the diameter of the fruit in inches was determined. Fruits that were to be picked in 1937 were first measured on September 25, 1936. At that time the average diameter of the measured fruits on each plot was 2 inches. They were remeasured fourteen times between then and July 8, 1937, when, again, the average diameters of the fruits on the wet and dry plots were the same, $2\frac{11}{16}$ inches.

The 1937–38 fruits were measured in a similar manner, except that 25 instead of 15 fruits on each of the same trees were measured. They were measured thirty-three times between June 17, 1937, and July 28,

^{14a} Between August, 1938, and September, 1940 (after this paper went to press), plots 3 to 6 received the same amounts of water, irrigations being made at the same time and as a part of the applications to the entire grove. On September 16, 1940, granulation tests were made on the plots. The results are of interest because they show that the "dry" plots (4 and 5) were again producing approximately the same amounts of granulation as the "wet" plots (3 and 6). For plots 4 and 5, the percentages were 16 and 13 in 1938, and 42 and 34 in 1940; while for plots 3 and 6, the percentages were 40 and 36 in 1938, and 43 and 41 in 1940.

1938. On these two dates the average diameters of the fruits on the plots were as follows:

Treatment and plot	Average diameter, in inches	
	June 17, 1937	July 28, 1938
Wet, plot 3	$\frac{1}{2}$	$2\frac{3}{8}$
Wet, plot 6	$\frac{1}{2}$	$2\frac{7}{16}$
Dry, plot 4	$\frac{1}{2}$	$2\frac{3}{8}$
Dry, plot 5	$\frac{1}{2}$	$2\frac{7}{16}$

The results of the fruit measurements show that, as conducted in this grove, the reduction in frequency of application and, consequently, in the amounts of water applied, had no appreciable effect on fruit size. The data also show that there was no appreciable difference in the growth rates of the fruits on the two sets of plots. On May 7, 1937, the approximate date of maturity of the 1936-37 fruit, the greatest difference in average diameter of the fruits on any two of the four plots was a little less than $\frac{1}{8}$ inch. One wet and one dry plot had the largest average sizes. The data for the fruit that came to maturity about May 5, 1938, showed similar results: the greatest average diameter difference this year was a little over $\frac{1}{16}$ inch; and, again, of the two plots on which the fruits had the largest average sizes, one was a dry plot and the other a wet plot. The plot differences were so small both years that they came well within the limits of experimental error.

The failure of the reduction in amounts of irrigation water to affect fruit sizes makes the granulation results more striking. While the fruits in granulation tests were not individually tagged and measured over the entire period, the sizes of the fruits picked at random for cutting were determined by using the sizing board (plate 2, A). Total yields per tree were obtained in 1937 and 1938; therefore, comparisons of yield, size, and granulation are made for these two years only.

In 1937, plot 4, infrequently irrigated over the entire period, yielded 11 field boxes of fruit more than the next highest-yielding plot, plot 6, which had been a wet plot for the entire period. Plot 5, which had been a wet plot from 1934 to 1936 and a dry plot from 1936 to 1938, yielded 3 field boxes of fruit more than plot 3, which had been a dry plot during the first period and a wet one during the latter period.

As already stated, 50 of the largest fruits from each tree were cut to determine granulation percentages in 1937, instead of 100 fruits chosen at random as in all other years. This was done to determine whether the reduction in the amount of water applied to certain plots had caused a reduction in fruit size. In making the determinations in 1937, it was found that of the fruits that were size 150, or larger, 15 per cent more came from plot 4 (dry) than from plot 6 (wet). The results for plots 3

and 5 were similar, but the differences were smaller: 3 per cent more came from plot 5 (dry) than from plot 3 (wet).

In 1938 the box yields were not determined, but the total number of mature fruits on each tree was counted on June 22. The two wet plots (3 and 6) produced almost the same number. The same was true for the two dry plots (4 and 5), but the two wet plots produced 13 per cent more fruits than the two dry plots. A part of this difference may be accounted for by the fact that more fruits dropped from the trees on the dry plots than from those on the wet plots before the counts were made.

For granulation determinations in 1938, 100 fruits were chosen at random from each tree. In running these fruits through the sizing board, it was found that plot 4 (dry) had 72 per cent more fruits that were size 150, or larger, than plot 6 (wet). The results for the other two plots were in reverse order, but the differences were not so great. Plot 3 (wet) had 40 per cent more fruits that were size 150, or larger, than plot 5 (dry).

Tests were made to determine whether the different irrigation treatments had caused appreciable differences in the soluble constituents of the juice of the fruit. On June 20, 1938, mature fruits were picked from each tree in each of the four plots and analyzed. The results showed that there was no appreciable difference in the total soluble solids, titratable acid, pH, and reducing and total sugars in the fruits from the wet and from the dry plots.

The general analysis of the irrigation experiment shows that the reduction in frequency of application and in amounts of irrigation water caused definite and marked decrease in the amount and severity of granulation. By 1938 there was a slight decrease in fruit yields but not in sizes of fruits from the dry plots. Of the 1,300 fruits from the wet plots and the 1,400 fruits from the dry plots (random sampling) cut for granulation tests in 1938, there were 18 per cent less fruits size 150, or larger, from the wet plots than from the dry plots. These results are of special interest because the largest fruits are usually those most likely to be granulated; yet the fruits from the dry plots, though averaging larger than those from the wet plots, showed a much lower percentage of granulation.

THE EFFECT OF THE ROOTSTOCK ON THE PRODUCTION OF GRANULATION

The possible relation between the rootstock and the prevalence of granulation was determined by testing the fruits from trees on the nine different rootstocks mentioned in table 7. The rootstock plots were in two different locations—at the Citrus Experiment Station, Riverside, and at the Irvine Ranch, Tustin, California. The rootstocks were planted in

replication in each plot.¹⁵ The fruits from the rootstock plots at Riverside were tested over a period of four successive years, 1935-1938, and those from the plots at Tustin, over a period of three years, 1936-1938. The fruits tested were from trees planted in 1927, with the following exceptions. At Riverside, 15 of the trees on Trifoliate-orange rootstock

TABLE 7

PERCENTAGES OF GRANULATED FRUITS FROM TREES ON DIFFERENT ROOTSTOCKS;
RIVERSIDE AND TUSTIN

Rootstock	Riverside					Tustin			
	1935	1936	1937	1938	Average	1936	1937	1938	Average
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
African sour orange, <i>Citrus aurantium</i> Linn.*	14	1.0	7.0	8	7.5	19	28	17	21.3
Rubidoux sour orange, <i>Citrus aurantium</i> Linn.*	24	1.0	7.0	12	11.0	13	28	19	20.0
Brazilian sour orange, <i>Citrus aurantium</i> Linn.*	10	1.0	12.0	8	7.8	34	37	32	34.3
Koethen sweet orange (1a4-24), <i>Citrus sinensis</i> Osbeck*	15	5.0	1.0	5	6.5	23	45	14	27.3
Sampson tangelo, <i>Citrus paradisi</i> Macf. × <i>C. reticulata</i> Blanco*	10	0.7	2.0	6	4.7	4	22	4	10.0
Cleopatra mandarin, <i>Citrus reticulata</i> Blanco*	7	1.0	3.0	10	5.3	22	37	19	26.0
Grapefruit (C.E.S. 343), <i>Citrus paradisi</i> Macf.*	4	0.2	0.4	7	2.9	19	33	17	23.0
Rough lemon, <i>Citrus limonia</i> Osbeck*	38	8.0	21.0	29	24.0	34	43	17	31.3
Trifoliate orange, <i>Poncirus trifoliata</i> (Linn.) Raf.:									
Trees planted 1927	39	24	32	31.7
Trees planted 1928	32	24.0	44.0	40	35.0	20	31	24	25.0
Trees planted 1929	45	19.0	42.0	52	39.5	23	24	66	37.7
Annual and total averages	20	6.1	13.9	18	14.4	23	32	24	26.1

* Trees planted in 1927.

were planted in 1928, and 10 of them in 1929. At Tustin, 10 trees on this rootstock were planted in each of the three years, 1927, 1928, and 1929. In making granulation tests, fruits were taken from 25 trees on Trifoliate-orange rootstock and from 10 trees on each of the other rootstocks in each locality. Twenty-five of the largest fruits were taken from both the north and the south half of each tree. Over the four-year period, 21,000 fruits were cut in the tests at Riverside and 15,750 in those at Tustin, or a total of 36,750 fruits in the two localities.

Table 7 gives a summary of the results obtained in the rootstock granulation tests at Riverside and Tustin. The results show that the trees on

¹⁵ These trees were propagated, planted, and grown under the direction of H. J. Webber, now Professor of Subtropical Horticulture, Emeritus, in the Citrus Experiment Station.

some rootstocks produced a much larger percentage of granulation than those on other rootstocks, and that granulation was much more prevalent in the plots at Tustin than in those at Riverside. At Riverside the Trifoliolate-orange and Rough-lemon rootstocks were noticeably the two highest producers of granulation. At Tustin the Brazilian sour orange was the highest in granulation production, the Trifoliolate orange (average of the three Trifoliolate plots) second, and the Rough lemon third. The lowest averages were shown by the C.E.S. 343 grapefruit (2.9 per cent) at Riverside and the Sampson tangelo (10 per cent) at Tustin.

Because of the low temperatures during the winter of 1936-37, four fruit conditions were encountered in the tests made at Tustin in 1937: healthy, granulated only, frozen only, and both granulated and frozen. There were no heaters in the rootstock plots at Tustin, but there were in the plots at Riverside. It is not easy to determine accurately whether a fruit is both frozen and granulated or only frozen, and for this reason the percentages of granulation given for Tustin in 1937 are probably a little too high. In the table it may be seen that the percentage of granulation for each of the rootstocks at Tustin, except the first and third Trifoliolate orange, was greatest in 1937. At Riverside only two rootstocks showed the greatest percentage of granulation in 1937.

The two Trifoliolate-orange plots at Riverside and the three similar plots at Tustin are listed separately in table 7 because they were planted in different years. As a rule, the younger the tree, the more granulation it is likely to produce. The data in the table show, however, that this did not always hold true for the fruits on Trifoliolate-orange rootstock, either at Riverside or at Tustin.

Conclusions drawn from the results obtained in the Riverside plots alone might be that at least certain rootstocks were an influencing factor in the production of granulation. The results from the plots at Riverside do not, however, entirely agree with those from the plots at Tustin. Comparison of the results obtained at both places leads to the conclusion that climatic conditions and other factors may be more important than rootstocks in governing the production of granulation.

Of the relation of rootstock to granulation, it is of interest to note that Bain writes as follows: "We find a rootstock connection with this trouble as at our station [British West Indies], we have plots on different stocks; the order of increasing severity is sour orange, Seville sweet, Rough lemon, wild grapefruit. There is not much difference between the latter two."¹⁶ And Lombard¹⁷ says that at the Rancho Sespe (Fillmore

¹⁶ Bain, F. M., Citrus Specialist, Department of Agriculture, Trinidad, British West Indies, in a letter to the senior author dated August 11, 1938.

¹⁷ Lombard, T. H., Assistant Manager, Rancho Sespe, Fillmore, California, in a letter to the senior author dated July 21, 1939.

California) a direct relation has been found between the kind of root-stock used and the percentage of granulated fruits produced, with severity increasing in the following order: sweet orange, sour orange, and Rough lemon.

GRANULATION AND LOW TEMPERATURES

Low temperatures are thought by some to have no effect on the amount of granulation produced; others think that low temperatures do not necessarily initiate granulation production, but that they augment it; while still others are of the opinion that low temperatures are the main cause of granulation. In the course of the studies on granulation, especially between the years 1932 and 1936, considerable evidence on the possible relation of temperature to granulation was accumulated (4). The fruit-cutting tests showed that many thousands of the fruits were granulated, slightly to severely, but not frozen; that many hundreds were frozen (segments collapsed), but not granulated; and, also, that many hundreds were both frozen and granulated. The evidence was still not conclusive; therefore during the picking seasons of 1936 and 1937, special studies were made to determine, if possible, whether there is any relation between low temperatures and granulation.

Mr. Young¹⁸ furnished records of the temperatures which prevailed in groves in which he had his weather instruments but in which there were no heaters. The records were obtained for the winters of 1935-36 and 1936-37.

Records on the percentages of granulation and freeze injury in the fruit for the 1936 season were secured, in most cases, from the packing-houses that handled the fruit from these groves. While these data were interesting and for the most part accurately taken, it was felt that each house might have a different standard for the determination of data. For this reason the results were not considered to be so reliable as if all of them had been obtained by the same person. Hence in 1937 the fruit samples from all the groves were cut and examined by one person. Each packing-house concerned sent one or two boxes of fruits from the grove from which the fruits were to be tested. The fruits were chosen by the man at the washer. As each box was dumped, a single fruit was chosen from it at random and placed in a special box. By this method a representative sample of the fruit from each grove was obtained. When the sample from a given grove had been collected, it was sent to the Citrus Experiment Station for immediate testing.

Each fruit was cut into four to six pieces. The results of the 1937 tests

¹⁸ Young, Floyd D., Senior Meteorologist, United States Weather Bureau, Pomona, California.

are recorded in table 8. They are especially significant because of the unusually low temperatures of the winter of 1936-37. The samples from every grove contained one or more frozen fruits. In the sample from one grove every fruit but one was frozen. The comparatively large number of fruits that were frozen but not granulated in most of the samples

TABLE 8
GRANULATION IN RELATION TO FROST INJURY, 1936-37

Grove no.	Temperatures to which fruits were exposed			Date fruits picked and cut, 1937	Total fruits in samples	Fruits healthy	Fruits frozen only	Fruits frozen and granulated	Fruit granulated only		
	Minimum	Time at 27° F or below	Time at 24° F or below						Slightly	Moderately	Badly
	°F	hours	hours		number	number	number	number	number	number	
2 a*	23.3	80¾	20¾	May 20	86	45	38	2	1	0	
9 a	23.5	18½	½	May 21	85	60	20	2	3	0	
5 a	23.2	55¾	1¼	May 24	150	60	88	1	1	0	
3 a	22.2	45¼	2¾	May 24	187	148	36	1	2	0	
41 a	22.2	34¾	13½	May 28	223	67	145	6	5	0	
23 w†	22.0	16¼	7	June 7	149	52	69	16	12	0	
42 w	22.7	48	5¾	June 9	125	96	6	0	21	2	
28 a	20.7	59½	16¾	June 11	159	77	78	3	1	0	
8 a	22.9	30	8¾	July 12	403	294	95	9	5	0	
39 a	24.6	33¾	0	July 16	167	28	137	2	0	0	
33 a	22.0	30	8¾	July 21	168	32	121	8	7	0	
20 a	19.2	30	9¾	July 23	269	7	253	9	0	0	
19 w	19.0	18¼	9¼	July 23	269	1	259	8	1	0	
26 w	20.0	30	11	Aug. 2	239	10	120	105	2	2	
12 w	23.7	47¾	5¼	Aug. 4	126	49	48	20	4	3	
7 a	24.0	23	2½	Aug. 16	98	24	14	16	23	14	
49 w	—	—	—	Aug. 19	175	79	66	19	10	1	
11 a	22.0	71½	6	Aug. 23	79	25	10	11	23	9	
41 a	22.0	65½	6	Aug. 23	124	21	55	43	3	2	
12 a	23.9	27¼	¼	Aug. 26	129	105	1	0	19	2	
35 a	24.0	8	1/12	Sept. 2	156	123	3	3	27	0	
22 w	22.0	26¼	3¼	Sept. 25	132	72	55	3	1	1	
27 w	—	—	—	Oct. 15	167	95	62	7	3	0	
Total	3,865	1,570	1,779	294	174	36	

* a = Groves located in Azusa district.

† w = Groves located in Whittier district.

may be noted in the table. Of the 3,865 fruits tested, 1,779 were in this condition. On the other hand, only 294 fruits were both frozen and granulated, and 222 granulated but not frozen.

The results confirm those obtained in the previous years. Low temperatures apparently do not cause granulation though they may augment it in those fruits which are inclined to granulate anyway. In such cases granulated juice sacs may be found in the midst of, or adjacent to, frozen tissues in any part of the fruit. There appears to be no evidence that low temperatures initiate granulation in fruits that do not have a tendency to granulate.

The data in table 8 show that granulation was more abundant during August, September, and October than during May, June, and July. This is in agreement with the generally accepted observation that granulation is much more prevalent in the late than in the early part of the picking season.

One of the most surprising things shown in table 8 is that in spite of the very low temperatures to which the fruits in the unheated groves were exposed, 1,570 of the 3,865 fruits showed no sign of injury and appeared to be in a healthy condition. Probably the fruit had become hardened by the several exposures to comparatively low temperatures during December and early January before the fruit-freezing temperatures came.

Concerning the possible effect of low temperatures on the production of granulation, the following comment from Bain is of interest:

With reference to the view that this trouble may be the result of freezing, I can assure you that there is not the slightest possibility of such a condition arising in Trinidad. The lowest temperature officially recorded in Trinidad for the past twenty years is 59° F. We are 10° N. of the equator and are therefore strictly tropical. While freezing might be a contributing factor in accentuating the trouble, it could not be primarily responsible, in view of our experience in Trinidad.¹⁹

Similar reports concerning the abundance of granulation in citrus fruits in Siam and Honduras, where the minimum temperatures are approximately the same as those in Trinidad, have been made verbally to the senior author by Boon-long²⁰ and Camp.²¹

THE EFFECT OF LIMB GIRDLING ON FRUIT SIZE AND GRANULATION

Limbs have been girdled to increase fruit size in some varieties of deciduous fruits. Whether limb girdling would cause the production of large citrus fruits was not known, but tests to determine this were made in 1936 and 1937. The main object of this experiment, however, was to determine what effect, if any, girdling might have on the amount and severity of granulation. Granulation is more often found in large than in small fruits; therefore, if girdling resulted in larger fruits, granulation should be more abundant.

On December 21, 1936, 1 limb on each of 22 trees was girdled. This procedure was repeated on different limbs of the same trees on March 11 and June 5, 1937, until 3 limbs on each tree, or a total of 66 limbs, had

¹⁹ Bain, F. M., Citrus Specialist, Department of Agriculture, Trinidad, British West Indies, in a letter dated August 11, 1938.

²⁰ Boon-long, T. S., a resident of Siam who was at the University of California Citrus Experiment Station during the summer of 1938.

²¹ Camp, A. F., Horticulturist in Charge, Citrus Experiment Station, Lake Alfred, Florida, who has made a study of citrus culture in Honduras.

been girdled. The diameter range of the different limbs at the point of girdling was from $\frac{9}{16}$ to $1\frac{1}{16}$ inches, but the diameters of most of the limbs were within the range of $\frac{5}{8}$ to $\frac{7}{8}$ inch. A ring of bark $\frac{3}{8}$ to $\frac{1}{2}$ inch wide was removed from each limb, the exposed wood was scraped to make sure that practically all cambium tissue had been removed, and the exposed surfaces were left bare. The fruits on these limbs were measured and tagged on the dates that the limbs were girdled. The fruits on limbs of similar size which branched from the same parent limbs were

TABLE 9
EFFECT OF LIMB GIRDLING ON FRUIT SIZE AND ON GRANULATION, 1936-37

Date of girdling and treatment of limbs	Fruits measured on date of girdling	Fruits tested on Sept. 9-10, 1937	Average diameter of fruits		Per cent granulated Sept. 9-10, 1937*
			On date of girdling	On Sept. 9-10, 1937*	
	<i>number</i>	<i>number</i>	<i>inches</i>	<i>inches</i>	<i>per cent</i>
December 21, 1936:					
Girdled.....	220	162	2.11	2.33	3.7
Nongirdled.....	220	185	2.20	2.38	2.2
March 11, 1937:					
Girdled.....	219	200	2.18	2.39	3.0
Nongirdled.....	219	200	2.25	2.40	3.5
June 5, 1937:					
Girdled.....	221	216	2.36	2.39	1.4
Nongirdled.....	222	215	2.36	2.40	1.4

* Dates when fruits were cut.

used as controls and were measured and tagged on the same dates as the test fruits. The fruits on the paired (girdled and nongirdled) limbs were remeasured and cut on September 9 and 10, 1937.

Table 9 shows the results of the girdling tests for 1937. There is no indication that the girdling caused an increase in size of fruit or that it had any effect on granulation. In general, the Valencias were small in the 1937 season, and the fruits on these branches were no exception. The fruits measured on December 21, 1936, and remeasured on September 9, 1937, increased in diameter only 0.22 and 0.18 inch, respectively, on the girdled and nongirdled limbs during that period. There was practically no growth in either case for fruits measured on June 5, 1937, and remeasured on September 10, 1937.

The girdling tests were carried over into the second year. The new crop of fruits from the same paired limbs were cut on September 13, 1938. This year the fruit diameters were not accurately measured as in the previous year, but their approximate sizes were determined by using the sizing board (plate 2, A). From each group (girdled and nongirdled

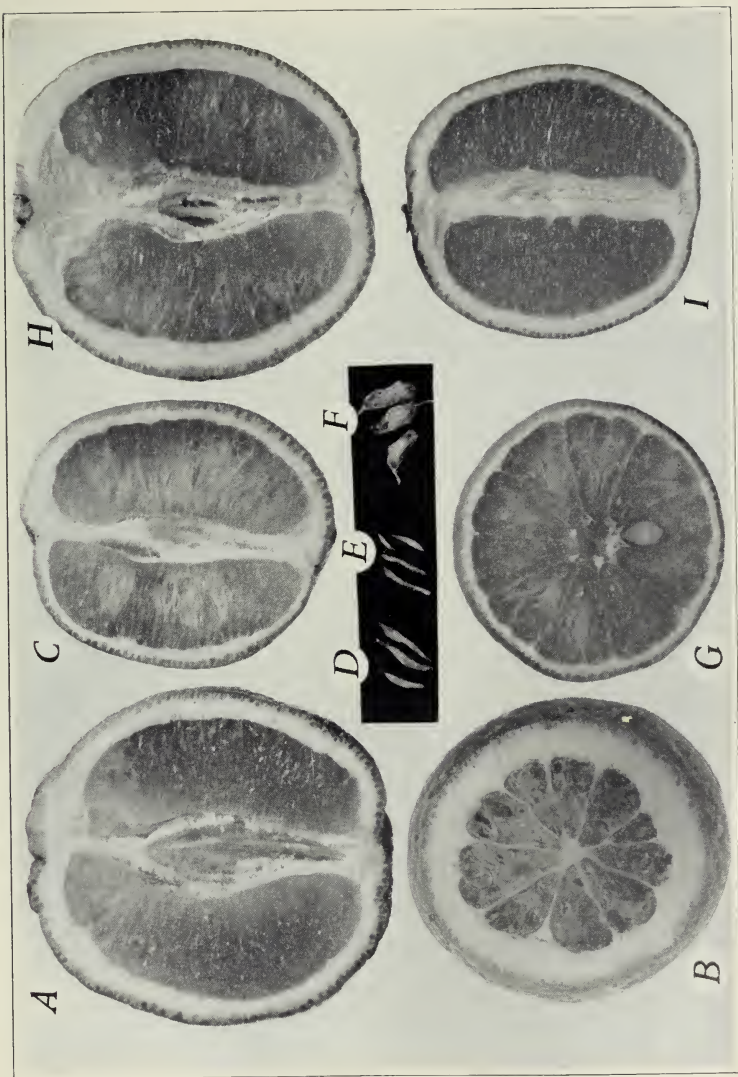
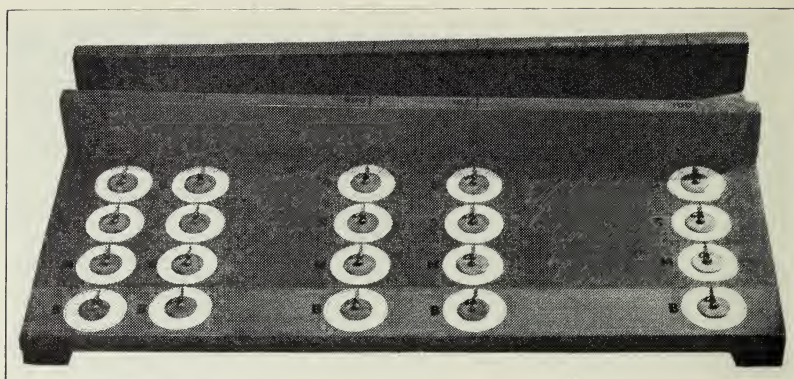
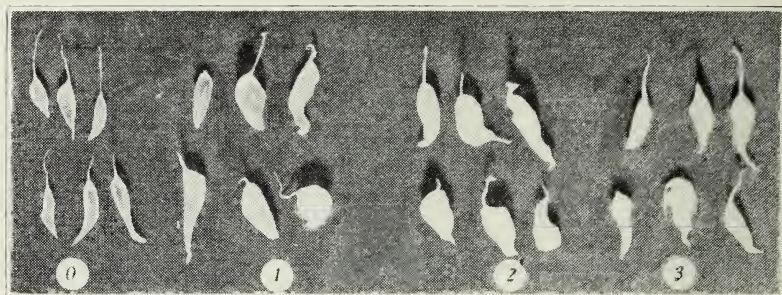


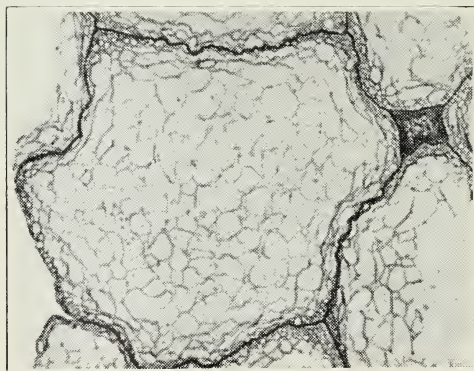
Plate 1.—Granulation and dry juice sac: A, B, longitudinal and transverse sections of granulated oranges; C, G, similar sections showing dry juice sac; H, longitudinal section showing both granulation and dry juice sac in the same fruit; I, healthy orange; D, E, F, dry, and granulated juice sacs. Note comparative sizes of D, E, and F.



A

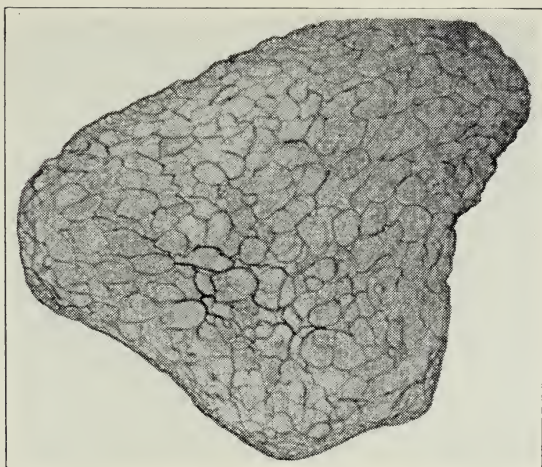


B

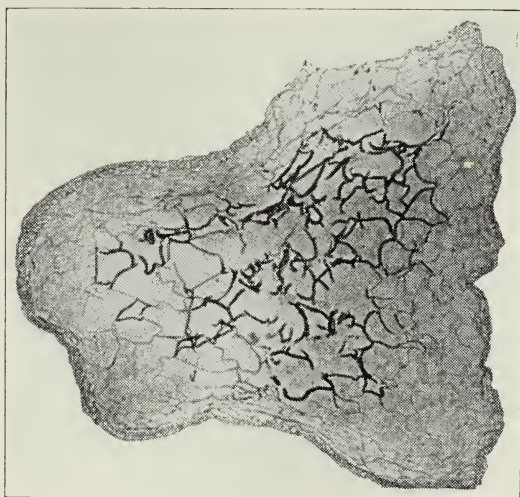


C

Plate 2.—A, Sizing and grading board used for determining and recording fruit sizes and for recording fruit quality with respect to granulation. The letters at the left of each row of dials refer to the quality of the fruit (G, good) or the severity of granulation (S, slight; M, moderate; and B, bad). (Courtesy of the *California Citrograph*.) B, Healthy and granulated juice sacs: 0, healthy; 1, 2, and 3, successive stages in granulation. (The process of granulation is progressive, and the stages indicated are purely arbitrary.) C, Enlarged cross section near the center of a healthy juice sac. Note the large number of comparatively thin-walled cells which compose the interior of the sac. (Compare with plate 3.) The dark rectangle at the right of the juice sac is a cross section of the stalk of a juice sac.

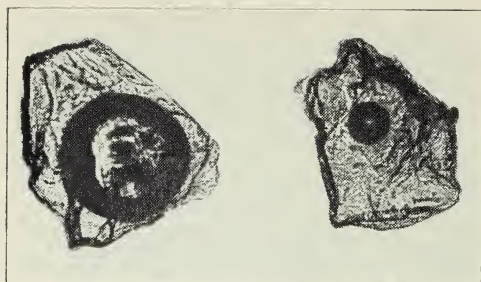


A

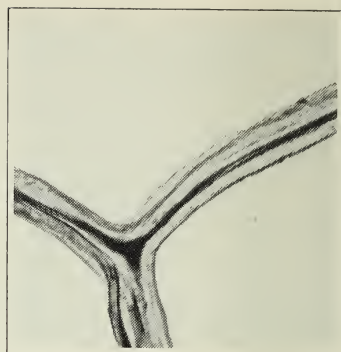


B

Plate 3.—Cross sections near the center of isolated juice sacs: *A*, showing some walls beginning to thicken because of granulation; *B*, showing the typical cell-wall thickening that occurs during granulation. Many of these walls have become lignified.



A



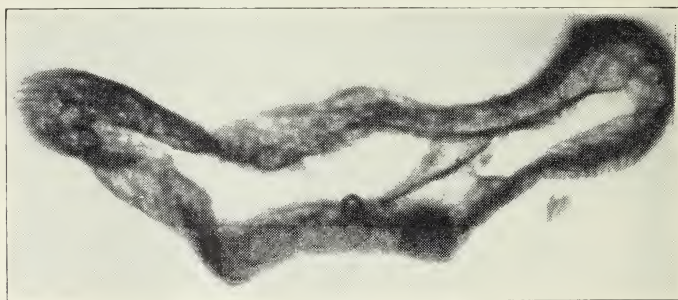
B



C



D



E

Plate 4.—*A*, Large thick-walled cells isolated from the interior of a granulated juice sac. The large dark rings are gas bubbles enclosed within the cells. The black dots on the surface are etched portions of the cell walls. *B*, Highly magnified cross section through the walls of three adjoining cells near the center of a granulated juice sac. The walls had become very much thickened, but etching had not yet begun. *C*, Longitudinal section through a thick-walled cell in a late stage of granulation, showing etching of cell walls, which results in wall perforations, the escape of the gas, and, finally, the disintegration of the entire cell. *D*, Longitudinal section through a portion of a juice sac in a late phase of stage 2 of granulation. Note the beginning of the central cavity, which is surrounded by large thick-walled cells, surrounded, in turn, by enlarged thin-walled cells. *E*, Freehand cross section through a juice sac in the final stage of granulation. Most of the enlarged thick-walled and thin-walled cells have disintegrated, and the sac has collapsed.

limbs), 521 fruits were cut. The summarized results of the tests are presented in table 10. Although a few of the girdled limbs bore especially large fruits, the average size of the fruits from these limbs was smaller than that of the fruits from the nongirdled limbs.

In 1938, 20 per cent of the fruits from the girdled limbs and only 5 per cent of those from the nongirdled limbs were granulated. This was

TABLE 10

EFFECT OF LIMB GIRDLING ON FRUIT SIZE AND ON GRANULATION, 1938
(Total number of fruits tested, 521 each from girdled and nongirdled limbs)

Size of fruit and treatment of limb	Number healthy	Number granulated			Total number of fruits	Percent fruits granulated
		Slightly	Moderately	Badly		
100's:						
Girdled.....	8	20	4	5	37	78
Nongirdled.....	11	4	0	0	15	27
150's:						
Girdled.....	48	38	4	5	95	49
Nongirdled.....	121	11	2	0	134	10
200's:						
Girdled.....	221	26	0	0	247	11
Nongirdled.....	333	7	1	0	341	2
288's:						
Girdled.....	142	0	0	0	142	0
Nongirdled.....	31	0	0	0	31	0
All sizes:						
Girdled.....	419	84	8	10	521	20
Nongirdled.....	496	22	3	0	521	5

unexpected because only 56 fruits on the girdled limbs were 150's or larger, while 132 fruits on the nongirdled limbs were similar sizes. Furthermore, the severity of granulation in the fruits on the girdled limbs was noticeably greater. Of sizes 100, 150, and 200 on the girdled limbs, the percentages of fruits granulated were 78, 50, and 11, respectively; percentages of fruits of similar sizes from the nongirdled limbs were 27, 9, and 2. None of the 288's were granulated.

GRANULATION IN RELATION TO SIZE OF FRUIT AND LOCATION ON TREE

Both size of fruit and location of fruit on the tree are important factors in the production of granulation. The importance of the location of fruit on the tree has been shown by the tests described on page 6, where the comparative percentages of granulation in fruits on five different parts of each of 10 trees are reported. Further comparisons of the

amounts of granulation in fruits on the north and south sides of the trees and in fruits of different sizes are shown in tables 11 and 12 and in columns 3 to 7, table 13. The figures in these tables were not obtained from experiments designed for this purpose alone but were compiled from the results obtained from most of the experiments already described.

TABLE 11

GRANULATION IN RELATION TO SIZE OF FRUITS AND LOCATION ON TREES, 1932-1938

Year and location of fruits on trees	Fruits, 150 per box and larger			Fruits, 200 per box and smaller			Fruits, all sizes	
	Number	Per cent*	Per cent granulated†	Number	Per cent*	Per cent granulated†	Number	Per cent granulated†
1932:								
North halves....	1,457	41	72	4,543	54	32	6,000	42
South halves....	2,100	59	67	3,900	46	29	6,000	42
1933:								
North halves....	2,504	53	91	2,996	48	69	5,500	79
South halves....	2,207	47	83	3,293	52	55	5,500	66
1934:								
North halves....	2,439	52	81	2,261	48	45	4,700	64
South halves....	2,230	48	49	2,470	52	22	4,700	35
1935:								
North halves....	811	49	58	539	52	28	1,350	46
South halves....	849	51	47	501	48	20	1,350	37
1936:								
North halves....	347	42	88	1,003	53	43	1,350	55
South halves....	470	58	59	880	47	19	1,350	33
1937:								
North halves....	527	48	50	148	60	17	675	43
South halves....	577	52	36	98	40	9	675	32
1938‡:								
North halves....	56	42	78	1,194	50	31	1,250	33
South halves....	76	58	49	1,174	50	15	1,250	17
Totals.....	16,650	25,000	41,650	..

* Based on total number for this size from both sides of the tree.

† Based on the total number granulated and healthy on each side of the tree separately.

‡ Fruit sizes were small in 1938. There were no 100's and comparatively few 150's.

Table 11 shows the comparative numbers of fruits, size 150 and larger and size 200 and smaller, from the north and south halves of the trees, from 1932 to 1938, inclusive. These data indicate that, except in 1933 and 1934, most of the larger fruits came from the south halves of the trees. In every case, however, granulation was most prevalent in the fruits from the north halves of the trees. They indicate, also, that the largest percentages of smaller fruits came from the north halves of the trees, except in 1933, 1934, and in 1938 (when the percentages from the

two halves were equal) ; and, again, that granulation was most prevalent in the fruits from the north halves of the trees. These results show that although, as a rule, the larger the fruit, the more likely it is to be granulated, yet the location of the fruit on the tree appears to be a more

TABLE 12
COMPARATIVE WEIGHTS OF FRUITS AND PERCENTAGES OF
GRANULATED FRUITS FROM NORTH AND SOUTH
HALVES OF TREES IN ROOTSTOCK PLOTS

Location of plots, year, and location of fruits on trees	Average weight of fruits*	Average percentage of fruits granulated
	<i>pounds</i>	<i>per cent</i>
Riverside:		
1935:		
North halves.....	101	25
South halves.....	112	15
1936:		
North halves.....	87	8
South halves.....	87	4
1937:		
North halves.....	93	18
South halves.....	96	9
1938:		
North halves.....	98	25
South halves.....	103	11
Tustin:		
1936:		
North halves.....	128	29
South halves.....	129	15
1937:		
North halves.....	117	39
South halves.....	122	25
1938:		
North halves.....	88	31
South halves.....	92	18
Averages:		
North halves.....	102	25
South halves.....	106	14

* Averages are based on the weights of 25 of the largest fruits from the north and south half of each tree in each rootstock plot.

important factor than the size. The information which this table contains was based on the cutting and observation of a total of 41,650 fruits.

The data in table 12 are based on weights of fruits rather than on numbers. Twenty-five of the largest fruits from both the north and the south half of each tree were weighed, and percentages of granulation were determined (the percentage being based on the total number granulated

and healthy from each half of the tree separately). The differences in weights of the fruits from the north and south halves of the trees were not great, ranging from no difference to a difference of 11 pounds; but, again, as shown by the data in table 11, no matter which half of the tree bore the larger fruits, the percentage of granulated fruits was greater from the north half.

The figures in tables 11 and 12 are averages. Some individual trees were exceptions to the general trend. But the high averages for the north

TABLE 13
COMPARATIVE PERCENTAGES OF FRUITS GRANULATED AND OF SEVERITY OF
GRANULATION, 1930-1938*

Year	Number of fruits picked and cut	Proportion of fruits granulated in various sizes					Severity of granulation, all sizes		
		100's	150's	200's	288's	344's	Slight	Moderate	Bad
1	2	3	4	5	6	7	8	9	10
	number	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1930†	3,820	—	—	—	—	—	55	30	15
1931†	6,425	—	—	—	—	—	58	29	13
1932	12,000	87	66	35	12	3	53	35	12
1933	11,000	97	88	67	37	18	35	39	26
1934	9,400	88	62	34	11	8	53	20	27
1935	2,700	91	68	39	10	0	52	31	17
1936‡	15,700	55	24	18	—	—	79	15	6
1937‡	8,500	64	29	23	—	—	67	21	12
1938‡	2,600	69	41	18	—	—	91	7	2
Averages or total	72,145	79	54	33	18	7	60	25	14

* Fruits came from 915 trees in 32 groves in Riverside, Orange, and Los Angeles counties.

† Dashes indicate that data were not available: fruit sizes were not determined in the 1930 and 1931 tests.

‡ Only the largest fruits were picked and cut in 1936, 1937, and 1938.

halves show that such exceptions were rare in the granulation percentages: in some instances the averages were twice or more than twice as high for the north as for the south halves of the trees, as is shown in the last column in table 12.

Table 11 indicates that granulation is much more prevalent in fruits of size 150 and larger than in those of size 200 and smaller. This is more strikingly apparent in table 13 (cols. 3-7). These results refer, of course, to typical stem-end granulation and not to freeze injury or dry sac, both of which may be confused with granulation by one who is inexperienced. The figures, which are averages, show very clearly that the larger the fruit, the more likely it is to be granulated. But there may be exceptions, for in at least occasional instances a fruit of size 200 from the north half of the tree was granulated, while a fruit of size 150 from the south half of the same tree was healthy.

SEVERITY OF GRANULATION

When a fruit was cut and examined, it was arbitrarily classed as healthy or as slightly, moderately, or badly granulated. Slightly granulated fruits come within the tolerance limit set for high-grade fruits; of the moderately granulated fruits, at least a large proportion can be shipped in an "off brand"; but badly granulated fruits have to be discarded or are fit for the products plant only.

The data in table 13 (cols. 8-10) show the comparative percentages of fruits that were slightly, moderately, and badly granulated over the period from 1930 to 1938. For the entire period, of the granulated fruits from 915 trees in 32 different groves, 60 per cent showed slight granulation, 25 per cent moderate, and 14 per cent bad. Results recorded for 1938 were obtained from a grove in which the fruits were small, and although many of them were granulated, the degree of severity was mainly slight. In some of the other years percentage figures would have been higher had it not been for the spray treatments²² and other factors which reduced not only the number of fruits granulated, but also the severity of granulation. For example, in 1932, 15 per cent of the fruits from the control trees and only 8 per cent of those from the sprayed trees were classed as badly granulated. Again, in 1936, one of the groves tested showed 23 per cent granulation that was classed as bad and another grove, 11 per cent; but in 10 other groves there was no fruit that could be so classified.

LABORATORY EXPERIMENTS

STAGES OF GRANULATION AND VOLUME OF JUICE SACS

During granulation, the juice sac goes through many progressive stages of change. For descriptive and analytical purposes, the visible changes have been arbitrarily grouped into stages 1, 2, and 3, or early, medium, and late stages. Healthy juice sacs, which in the following discussion will be referred to as stage "0," and juice sacs in stages 1, 2, and 3 of granulation are shown in plate 2, *B*.

The juice sacs begin to increase in size as soon as granulation sets in and reach their maximum size in stage 2. There is a gradual decrease in volume in stage 3, though even in this stage they remain, as a rule, a little larger than healthy sacs. In plate 2, *B*, the sacs designated as in stage 3 appear to be almost as large as those in stages 1 and 2, but they were, in reality, collapsed and more or less flat. These conditions refer to typically granulated juice sacs as found in the stem-end fourth of the

²² Spray treatments affected the severity of granulation, but, so far as could be determined, did not affect its distribution among fruit sizes. Data from both sprayed and unsprayed trees are therefore included in table 13.

fruit pulp. The comparative volumes of healthy and granulated juice sacs are shown graphically in figure 3. Determinations are based on measurements of about 400 juice sacs in each stage.

Further and more detailed information on the biochemical, structural,

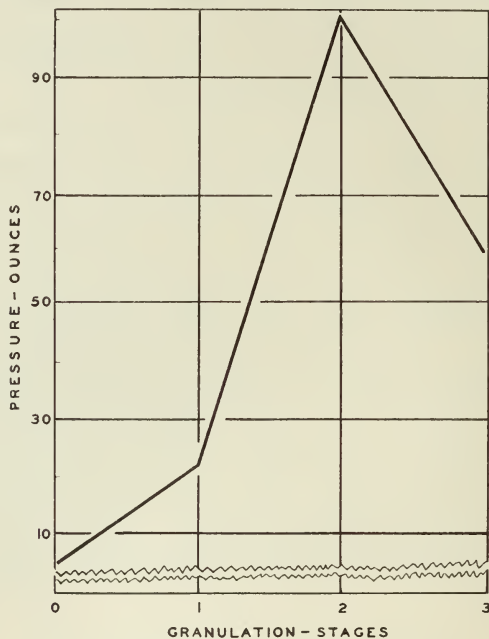


Fig. 3.—Comparative volumes of healthy juice sacs and those in different stages of granulation. Determinations are based on measurements of about 400 juice sacs in each stage.

and other changes which occur in the juice sacs during the process of granulation have been described by Bartholomew, Sinclair, and Raby (2), Turrell and Bartholomew (8), and by Schroeder.²⁸

NITROGEN IN JUICE SACS

A study was made of the comparative amounts of nitrogen, as determined by the Kjeldahl-Gunning method, in healthy and in granulated juice sacs. The fruit samples came from different groves. The healthy sacs were taken in mass from the stem-end third of five different, healthy oranges. The granulated sacs (stages 1 and 2) were individually isolated from the same number of granulated fruits from the same grove. The whole juice sacs, both juice and tissues, were used in making the deter-

²⁸ Schroeder, C. A. Histological studies of citrus fruit vesicles. Thesis for the degree of Master of Science, University of California, 1936. (Typewritten.) Copy on file in the Library of the University of California at Los Angeles.

minations. The results are given in table 14 and show that under these conditions there was no appreciable difference between the amounts of nitrogen in the healthy and in the granulated juice sacs. While nitrogen undoubtedly plays an important part in the physiological activity of the juice sac, it is of interest to find that the amount present is so small.

TABLE 14
COMPARATIVE PERCENTAGES OF NITROGEN IN HEALTHY AND IN
GRANULATED JUICE SACS*
(Fresh-weight basis)

Sample no.	Nitrogen in juice sacs	
	Healthy	Granulated
	<i>per cent</i>	<i>per cent</i>
10.....	0.16	0.15
11.....	.12	.13
16.....	.14	.16
17.....	.12	.16
18.....	.12	.16
19.....	.17	.13
20.....	.14	.14
21.....	.16	.14
Average.....	0.14	0.15

* Each pair of samples (healthy and granulated juice sacs) was taken from a different grove.

REDUCING AND TOTAL SUGARS AND TOTAL SOLUBLE SOLIDS

Determinations were made of the comparative amounts of sugars in apparently healthy and in granulated juice sacs individually isolated from the stem-end fourth of the same fruits. The term "healthy" should be qualified. When the so-called "healthy" sacs were obtained from affected fruits, they were lying adjacent to or near sacs that were granulated. They appeared to be healthy, but that they were not entirely so will be indicated in some of the following sections. In spite of the possibility that these sacs were not entirely healthy, it seemed advisable, in most cases, to use them for comparative purposes rather than sacs from some other portion of the same fruit or from the same portion of fruits that were not granulated. Juice sacs obtained from nongranulated fruits were taken from the stem-end fourth because the granulated sacs were taken from that portion of granulated fruits. This procedure was followed because other workers (1, 6, 7) had found that fruits show individual differences and that there is usually a gradient or polarity of substances, principally sugars, in citrus fruits toward the stylar end.

Each sample consisted of a known weight of juice sacs (approximately 25 grams) from 5 to 12 oranges. The number of fruits used depended

upon how severely they were granulated. The reagents were standardized against pure glucose obtained from the United States Bureau of Standards. Determinations were made during the summers of 1933,

TABLE 15
PERCENTAGES OF REDUCING AND TOTAL SUGARS IN "HEALTHY" AND IN GRANULATED JUICE SACS*

Year and sample† no.	Reducing sugars		Total sugars	
	"Healthy" sacs	Granulated sacs	"Healthy" sacs	Granulated sacs
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1933:				
1.....	4.43	3.28	9.69	5.00
2.....	4.44	3.22	8.71	5.01
3.....	4.49	3.21	8.61	4.84
4.....	4.47	3.23	9.55	4.84
5.....	4.49	3.10	8.61	4.65
6.....	4.21	3.16	9.06	4.66
7.....	4.24	3.11	9.01	4.75
8.....	4.22	3.18	9.03	4.76
Average.....	4.37	3.19	9.03	4.81
1934:				
9.....	3.02	2.25	6.93	3.17
10.....	3.65	2.03	6.14	3.14
11.....	2.96	1.78	6.10	2.86
12.....	2.68	1.96	5.76	3.33
13.....	3.34	2.09	6.23	2.99
14.....	3.63	2.26	6.18	3.32
Average.....	3.21	2.06	6.22	3.14
1935:				
15.....	2.06	1.99	5.47	4.00
16.....	2.47	1.45	5.41	3.35
17.....	1.99	1.81	5.53	3.87
18.....	1.73	1.98	5.84	3.77
19.....	1.80	1.28	5.27	3.76
20.....	2.17	1.03	5.01	3.70
Average.....	2.04	1.59	5.42	3.74
Three-year average.....	3.21	2.28	6.89	3.90

* Percentages are based on fresh weights and are expressed in terms of glucose. For explanation of "healthy," see text, p. 41.

† Each sample came from a different grove and consisted of approximately 25 grams of individually isolated juice sacs; both juice and juice-sac tissue were used in tests.

1934, and 1935; and the results, based on fresh weights of samples and expressed in terms of glucose, are presented in table 15.

The data in table 15 show very plainly that the granulated juice sacs contained considerably lower concentrations of sugars than the adjacent "healthy" sacs from the same fruits. The decrease in concentration of total sugars was greater than that of reducing sugars. For the entire

three-year period, the amount of total sugars in the granulated juice sacs averaged only 57 per cent and for reducing sugars alone, 71 per cent of that in the "healthy" sacs.

The amounts of reducing sugars in the "healthy" juice sacs were greater than those in the granulated sacs, except in sample 18, 1935. The amounts of total sugars were always greater in the "healthy" sacs. The average amounts of sugars in both kinds of juice sacs varied considerably in different years. In 1933 the average for the total sugars in the "healthy" sacs was 9.03 per cent, which is only a little under the average for whole, mature, healthy Valencia oranges at the time of the year at which these tests were made (July). In 1934 and 1935, however, the averages for the total sugars in the "healthy" juice sacs were only 6.22 and 5.42 per cent, respectively. The amounts of sugars present in the juice sacs are, of course, largely governed by the condition of the fruit at the time the tests are made; but a large number of tests in these and related experiments show that whole, mature, healthy Valencia oranges at this time of the year contain from 7 to 10 per cent total sugars. These results indicate that although the juice sacs were designated as "healthy," they were not entirely healthy. They, nevertheless, contained considerably higher concentrations of sugars than juice sacs that were visibly granulated.

The data recorded in table 15 show the comparative concentrations of sugars in both juice and tissues of "healthy" and granulated juice sacs. In the next series of tests, juice sacs of both kinds were individually isolated and segregated as before; but in this case only the juice was tested, not for sugars alone, but also for total soluble solids, titratable acidity, and pH. In most of these tests, sugar determinations were made separately on juice sacs that were apparently healthy and on those that were in stages 1 and 2 of granulation (plate 2, *B*). Approximately 80 grams of each kind of juice sac was used in each sample. The sacs were thoroughly ground in a mortar in the presence of clean quartz sand, strained, and the juice was centrifuged. The total soluble solids were determined by the use of an Abbé refractometer instead of by the Brix method. The results of these tests are presented in table 16.

The comparative concentrations of total and reducing sugars in these tests show the same relation with reference to the "healthy" and granulated condition as was shown in the immediately preceding tests, where concentrations were based on the fresh weight of both juice and tissues of "healthy" and granulated juice sacs. In general, the concentration of sugars decreased as the severity of granulation increased. In every case the juice from the "healthy" sacs contained a higher concentration of total sugars than that from the granulated sacs. While the reducing-

TABLE 16
COMPARATIVE ANALYSES OF JUICE FROM "HEALTHY" AND GRANULATED
JUICE SACS*

Sample no. and type of juice sac†	Total soluble solids	Citric acid	Active acidity	Soluble solids as glucose	
				Reducing	Total
	<i>per cent</i>	<i>per cent</i>	<i>pH</i>	<i>per cent</i>	<i>per cent</i>
No. 1:					
"Healthy".....	9.18	0.59	3.95	3.50	6.74
Granulated—1.....	7.44	.37	4.45	3.08	5.36
Granulated—2.....	7.31	.32	4.65	3.14	4.98
No. 2:					
"Healthy".....	9.44	.60	4.00	3.72	7.68
Granulated—1.....	7.58	.34	4.50	3.29	5.66
Granulated—2.....	7.18	.27	4.65	2.91	5.10
No. 3:					
"Healthy".....	9.11	.55	4.10	3.53	6.82
Granulated—1.....	7.18	.38	4.40	2.99	5.33
Granulated—1.....	7.24	.36	4.45	2.99	5.21
Granulated—2.....	7.31	.30	4.63	3.02	5.05
No. 4:					
"Healthy".....	9.31	.64	3.90	3.31	6.96
Granulated—1.....	6.51	.30	4.60	2.76	4.77
Granulated—2.....	6.31	.31	4.70	2.69	4.16
No. 5:					
"Healthy".....	8.91	.58	4.00	3.26	6.79
Granulated—1.....	6.64	.35	4.55	2.76	4.77
No. 6:					
"Healthy".....	8.64	.61	4.00	3.32	6.56
Granulated—1.....	6.71	.38	4.43	2.76	4.61
Granulated—2.....	6.44	.33	4.60	2.80	4.17
No. 7:					
"Healthy".....	7.84	.50	4.10	2.75	5.68
Granulated—1.....	6.78	.34	4.60	2.84	4.61
No. 8:					
"Healthy".....	7.98	.70	3.85	2.91	5.97
Granulated—1.....	7.04	.37	4.40	2.99	4.99
Granulated—2.....	6.71	.30	4.60	2.84	—
No. 9:					
"Healthy".....	8.64	.58	4.00	3.05	6.56
Granulated—1.....	7.51	.35	4.55	3.14	5.21
No. 10:					
"Healthy".....	8.44	.75	3.95	3.05	5.73
Granulated—1.....	7.04	.41	4.45	2.92	4.61
Granulated—2.....	6.51	.32	4.65	2.77	4.12
No. 11:					
"Healthy".....	7.84	.55	4.00	2.76	5.93
Granulated—1.....	6.71	.38	4.50	2.83	4.61
Average:					
"Healthy".....	8.67	.60	3.20	6.49
Granulated—1.....	7.03	.36	2.95	4.98
Granulated—2.....	6.82	0.31	2.88	4.60

* Each sample came from a different grove and consisted of approximately 80 grams each of "healthy" and granulated (individually isolated) juice sacs. For explanation of "healthy" see text, p. 41.

† "Granulated—1" (stage 1) indicates a less advanced stage of granulation than "granulated—2" (stage 2). See plate 2, B.

sugars average was highest for the juice from the "healthy" sacs, there were four individual exceptions: samples 7, 8, 9, and 11.

It should be pointed out again that the juice sacs designated as "healthy" in these tests were not entirely so, and that if juice from sacs from the styler end of these fruits, or from nongranulated fruits, had been used for comparison, the results would have been more striking. In all cases, the concentrations of total sugars were higher in stage 1 than in stage 2 of granulation. Four of seven tests showed a higher concentration of reducing sugars in stage 1 than in stage 2.

The juice from the "healthy" sacs was noticeably higher in total soluble-solids content than that from the granulated sacs: the average for the juice from the "healthy" sacs was 8.67 per cent, and that for juice from the granulated sacs (stages 1 and 2) 6.93 per cent. With one exception (sample 3), the total soluble-solids content of the juice from the granulated-1 sacs was higher than that from the granulated-2 sacs.

TITRATABLE ACIDITY AND pH

The comparative amounts of total acidity (titratable with standard sodium hydroxide) and active acidity (pH) in healthy and granulated juice sacs was determined from an aliquot of the same juice that was used in determining the total soluble solids and sugars, as described in the preceding section. The results of these determinations are recorded in table 16. The data show that the titratable acidity, expressed as percentage of citric acid, decreased with the advancement of granulation and that there was a corresponding decrease in active acidity, as indicated by the higher pH readings. It is permissible to express titratable acidity in terms of citric acid because no other organic acid is present in the juice sacs in appreciable quantities.

In every one of the eleven samples tested (table 16), the "healthy" juice sacs contained a higher concentration of citric acid than those that were granulated, and the granulated-1 sacs contained more acid than the granulated-2 sacs. The average of all tests showed a titratable acidity of 0.60 per cent for the juice from the "healthy" sacs and of 0.36 and 0.31 per cent for the granulated-1 and granulated-2 sacs, respectively. The average titratable acidity of nongranulated Valencia oranges at the time these tests were made (latter part of July) was about 0.9 to 1.0 per cent.

TOTAL PECTIN AS CALCIUM PECTATE

Studies were made on the comparative amounts of pectic substance in healthy and in granulated juice sacs. The healthy sacs were not isolated separately, but were taken in small masses from the pulp near the stem end of nongranulated fruits. The granulated juice sacs (stage 2) were

isolated separately and taken from near the stem end of granulated fruits. In each test the healthy and granulated sacs came from the same lot of fruits. The entire juice sac and its contents were used in each case. All segment-wall tissues were excluded.

The results of these tests are shown in table 17. They are based on fresh weight and represent the total pectin content, soluble and insoluble, in terms of calcium pectate. The size of the sample used in each

TABLE 17
PERCENTAGES OF TOTAL PECTIN AS CALCIUM PECTATE IN
HEALTHY AND GRANULATED JUICE SACS*
(Fresh-weight basis)

Experiment no.	Healthy juice sacs	Granulated juice sacs (stage 2)
	<i>per cent</i>	<i>per cent</i>
1.....	0.32	0.60
2.....	.30	.62
3.....	.34	.66
4.....	0.31	0.60

* The entire juice sac and its contents were used in each test. All segment-wall tissues were excluded.

determination was governed by the amount necessary to yield approximately 30 mg of calcium pectate. The results show that there was approximately twice as much pectic material in the granulated (stage 2) as in the healthy juice sacs.

Studies are now being made to determine the different forms and comparative amounts of pectin in juice sacs that are in different stages of granulation and in those that are healthy.

MOISTURE CONTENT OF JUICE SACS

The comparative moisture contents of apparently healthy and granulated juice sacs were determined during the seasons of 1935 and 1936 from granulated juice sacs in the stages designated as 1 and 2 (plate 2, *B*) and from adjacent nongranulated sacs in the stem-end fourth of the same fruits. The segregated juice sacs (25 to 30 grams) were put into glass weighing bottles, weighed, and placed in an oven at 100° C for 1 hour to destroy enzyme action. They were then brought to constant weight in a vacuum oven. The results of some of these tests have been partially reported elsewhere (2), and only a small, representative number of the tests will be presented here (table 18).

Comparatively little juice can be reamed from badly granulated tissues, but the data in table 18 indicate that the granulated juice sacs actually contained a higher percentage of moisture than those that were

not granulated. The moisture is no longer in liquid form in the granulated sacs, but is apparently physically or otherwise bound in the walls and other portions of the sac, so that it cannot be reamed out as juice. Turrell and Bartholomew (8) have shown that in a later stage (stage 3,

TABLE 18
COMPARATIVE PERCENTAGES OF MOISTURE IN "HEALTHY" AND
GRANULATED JUICE SACS*
(Fresh-weight basis)

Sample no.	"Healthy" juice sacs	Granulated juice sacs	Difference (granulated— "healthy")
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1.....	90.83	92.26	1.43
2.....	90.39	92.62	2.23
3.....	90.14	91.32	1.18
4.....	91.48	92.78	1.30
5.....	90.94	92.46	1.52
6.....	91.04	92.46	1.42
Average.....	90.80	92.32	1.52

* Samples of individual "healthy" and granulated juice sacs were isolated and tested separately. For explanation of "healthy," see text, p. 41.

juice sacs collapsed) the granulated sacs contain just about the same percentage of moisture as those lying adjacent but not visibly granulated, or possibly a little less.

DRY MATTER AND INORGANIC CONSTITUENTS

Tests for determining the comparative amounts of dry matter and inorganic constituents of healthy and granulated juice sacs were made during the seasons of 1935 and 1936. Each sample came from a different grove (from a lot of 25 fruits) and included entire juice sacs and their contents. In the first four tests, apparently healthy and granulated juice sacs (stages 1 and 2) were isolated from the stem-end fourth of the same fruits. In the remaining tests, the granulated sacs were individually isolated from a similar number of granulated fruits, but for comparison, a similar quantity of healthy tissue was taken from the stem-end fourth of nongranulated fruits from the same locality. The fresh weight of each lot of juice sacs was carefully determined.

The results of these tests are presented in table 19. They show that the dry matter was always considerably higher in the healthy than in the granulated juice sacs. The differences range from 0.81 to 2.97 per cent. On an average there was 1.77 per cent more dry matter in the healthy and apparently healthy juice sacs than in the granulated ones.

In another test, over 1,000 juice sacs were individually isolated and segregated into stages 0 (healthy) and 1, 2, and 3 (granulated). Two

TABLE 19
 DRY MATTER AND INORGANIC CONSTITUENTS OF HEALTHY AND GRANULATED
 JUICE SACS*

Year, sample no. and type of juice sac	Dry matter, fresh- weight basis	Ash		Inorganic constituents, dry-matter basis				
		Fresh- weight basis	Dry- weight basis	Calcium	Magne- sium	Potas- sium	Sodium	Phos- phate
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1935:								
No. 1:								
"Healthy"†.....	9.17	0.447	4.88	0.13	0.18	1.88	0.90	0.40
Granulated.....	7.14	.605	7.77	.37	.23	2.83	.98	.64
No. 2:								
"Healthy"†.....	9.61	.378	3.93	.18	.14	1.39	.67	.50
Granulated.....	7.38	.569	7.70	.35	.28	2.69	.87	.69
No. 3:								
"Healthy"†.....	9.06	.488	5.38	.18	.17	2.24	.64	.61
Granulated.....	7.54	.611	8.11	.25	.23	3.08	.99	.61
No. 4:								
"Healthy"†.....	9.22	.442	4.77	.15	.13	1.86	.35	.74
Granulated.....	8.03	.629	7.83	.24	.20	2.95	.68	.69
1936:								
No. 5:								
Healthy.....	8.52	.481	5.65	.19	.12	2.17	.15	.35
Granulated.....	7.22	.612	8.48	.39	.19	3.51	.22	.47
No. 6:								
Healthy.....	8.82	.458	5.20	.12	.12	2.19	.12	.30
Granulated.....	7.22	.622	8.65	.41	.20	3.67	.23	.54
No. 7:								
Healthy.....	8.68	.509	5.86	.15	.15	2.32	.15	.37
Granulated.....	7.87	.678	9.19	.40	.25	3.39	.28	.52
No. 8:								
Healthy.....	10.78	.437	4.06	.11	.11	1.69	.14	.23
Granulated.....	7.97	.596	7.48	.29	.21	3.11	.83	.38
No. 9:								
Healthy.....	10.20	.475	4.68	.12	.11	1.85	.25	.32
Granulated.....	8.30	.610	7.36	.34	.22	3.00	.91	.38
No. 10:								
Healthy.....	10.85	.386	3.56	.14	.10	1.79	.26	.21
Granulated.....	7.88	.571	7.26	.20	.19	2.98	.36	.38
Average, both years:								
Healthy.....	9.49	.450	4.80	.15	.13	1.94	.36	.40
Granulated.....	7.66	0.610	7.98	0.32	0.22	3.12	0.64	0.53

* Each sample came from a different grove and consisted of 80 grams each of healthy and granulated (stages 1 and 2) juice sacs from 25 fruits. Groves were in three different localities—Garden Grove, Santa Ana, and Riverside.

† For explanation of "healthy" in the 1935 tests, see text, p. 41.

lots of juice sacs in each stage were tested in each of two different years. The granulated sacs were taken from near the stem end and the healthy sacs from near the center of the same fruits. The results for the two years were similar, and the graph, figure 4, records the average for the four tests for each of the four stages. These results are based on the average dry weight per individual juice sac and not on equal weights of healthy and granulated sacs.

Table 19 shows that when dry matter was based on *equal weights* of

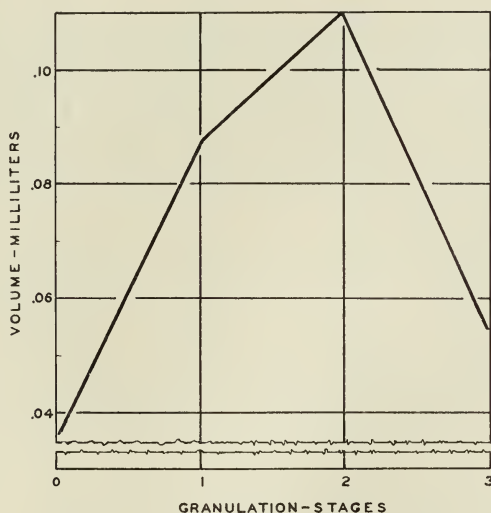


Fig. 4.—Comparative amounts of dry matter in juice sacs in stages 0 (healthy) and 1, 2, and 3 (granulated). Determinations were based on average dry weight per individual juice sac.

juice sacs—that is, on a percentage basis—the dry matter was 1.83 per cent higher in the healthy than in the granulated juice sacs.

When the dry weight was based on amount of increase per *individual juice sac* (fig. 4), the differences were reversed and were much more marked, which indicates that there had been an actual growth of the juice sacs during the early stage of granulation. The increase in dry weight in stage 1 over stage 0 was 206 per cent. When equal weights of these particular healthy and granulated juice sacs (stages 1 and 2) were compared, rather than individual sacs, it was found that the excess of dry matter in the granulated sacs over the dry matter in the healthy sacs was similar to that shown in table 19, but a little greater. Small differences were to be expected because the samples of fruit came from different localities.

The segregation of the granulated juice sacs into stages 1, 2, and 3

was, of course, arbitrary; therefore the differences in dry weight between stages 1 and 2, and between stages 3 and 0 as shown in the graph (fig. 4) may not be significant. The main points of interest are the great increase in dry matter in stages 1 and 2 and the sharp decrease in stage 3.

In a succeeding section (see "Juice-Sac Collapse," p. 52) it will be shown that the granulated sac begins to disintegrate during stage 2, as is shown by the accumulation of gas in certain internal cells. This disintegration may account for the loss of dry matter by the time stage 3 has been reached.

The amount of ash, on a basis of both fresh and dry weight, was always lower in the healthy than in the granulated juice sacs (table 19). The differences were fairly consistent, ranging, on a fresh-weight basis, from 0.123 to 0.191 per cent, and on a dry-weight basis from 2.68 to 3.77 per cent. On an average, there was 0.16 per cent more ash on a fresh-weight basis and 3.18 per cent on a dry-weight basis in the granulated than in the healthy juice sacs.

A study of table 19 will also show that the principal inorganic constituents were highest in the ash from the granulated juice sacs. This was true for calcium, magnesium, potassium, and sodium, and in most cases for phosphate. Similar tendencies have been reported by Haas and Klotz (6) in connection with their work on gradients in citrus fruits. The differences for calcium and magnesium are especially large, there being an average of only 46.88 per cent as much calcium and 59.09 per cent as much magnesium in the healthy juice sacs as in the granulated ones. The sodium values for the season of 1935 (samples 1 to 4) were higher than those for 1936 (samples 5 to 10). The fruit from which the samples were taken came from three different localities, and it is probable that the trees from which the 1935 samples were taken had access to comparatively large amounts of sodium. The form in which these several elements existed in the two kinds of juice sacs tested will not be discussed here. The significant and interesting thing about them is that they accumulated in excess in the granulated sacs.

STRUCTURAL AND OTHER CHANGES IN JUICE SACS DURING GRANULATION

Enlargement of Juice Sacs.—That the juice sacs enlarge during the process of granulation is indicated by the fact that a volume equal to 16.4 cubic centimeters (1 cubic inch) required approximately 475 healthy sacs, whereas the same volume required only approximately 213 sacs in stage 1, 146 sacs in stage 2, and 295 sacs in stage 3 of granulation. The comparative sizes of healthy sacs and of those in the three stages of granulation are shown graphically in figure 3 (p. 40). As mentioned be-

fore, some of those in the collapsed condition (plate 2, *B*, stage 3) appear to be larger than they really are because of the position they were in when photographed. They may be broad but are comparatively thin (plate 4, *E*).

Hardening of Juice Sacs and Thickening of Cell Walls.—Evidence that juice sacs harden as well as enlarge as they become granulated is shown by figure 5. In making these determinations, individual juice sacs

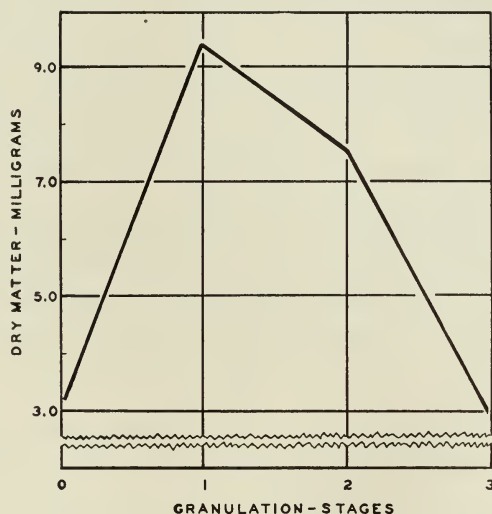


Fig. 5.—Comparative pressures (in ounces) necessary to crush healthy (stage 0) juice sacs and those in stages 1, 2, and 3 of granulation to 1 mm in thickness.

were tested, and pressure was applied gradually until the sac had been crushed to a thickness of 1 mm. A pressure of approximately 140 grams (5 ounces) was found to be sufficient to crush the average healthy juice sac, while to crush a sac that was in the early or medium stage of granulation took from 500 to 3,000 grams (20 to 100 ounces) or more. In stage 1 of granulation, the hardness appears to be due principally to the fact that the juice in the cells of the sac becomes more or less gelatinized. In stage 2 this condition persists in some of the cells, but what is more significant is that the walls of some of the internal cells may become as much as twenty-five times as thick as they were before the sac began to granulate.

Photomicrographs of cross sections of healthy and granulated juice sacs are shown in plates 2, *C*, and 3, *A*, *B*. Plate 2, *C*, shows that each healthy juice sac is composed of numberless small, thin-walled cells and is not merely a single cell or a little bag filled with juice, as it is so often,

erroneously, thought to be. Each of the small cells that make up the interior of healthy juice sacs remains intact and alive, even after picking, so long as the fruit is not destroyed.

The walls of the cells which compose the interior of the healthy juice sac are very thin, and for that reason it is impossible to tease out or isolate a single cell. When the walls of some of these cells have become thickened and hardened, as illustrated in plate 3, *B*, it is not difficult to isolate the cells with dissecting needles under low magnification. Plate 4, *A*, is a photomicrograph of two thick-walled cells isolated from the interior of a granulated juice sac and many times larger than any of the cells in healthy juice sacs. The large dark rings shown within these cells are internal gas bubbles. The black dots on the surface indicate the beginning stages of cell-wall etching, which finally results in the escape of the gas and the disintegration of the cell.

Plate 4, *B*, is a photomicrograph of a cross section through the walls of three adjoining cells near the center of a granulated juice sac. The walls had become very much thickened, but etching had not yet begun. The dark line between the walls of the adjoining cells represents the material which holds the walls together. It contains a comparatively large amount of calcium pectate.

Plate 4, *C*, is a photomicrograph of a longitudinal section through an enlarged, granulated cell whose contents have disappeared and whose wall has become very much etched.

Juice-Sac Collapse.—The final stage of granulation (stage 3) is typified by a softening and collapse of the juice sac, caused by the progressive disintegration of the cells, which begins near the center of the juice sac. Plate 4, *D*, shows the beginning of the formation of the central cavity. The collapse begins at about this stage and continues, as more of the cells disintegrate, until the sac becomes flattened or more or less angular, its form depending upon the directions of lateral pressure. Plate 4, *E*, is a photomicrograph of a cross section through a collapsed granulated juice sac.

Under certain conditions it is not easy to distinguish between frozen and granulated collapsed juice sacs. The collapsed granulated sacs, however, usually have less color than similar frozen sacs, and microscopic examination shows that the granulated sacs have an uneven or roughened appearance on the surface, which the frozen sacs do not have. The uneven appearance is caused by the presence of some of the thick-walled, not-yet-disintegrated cells just inside the juice-sac wall.

Lignification of Cell Wall.—As has been stated, the early stages of granulation are characterized by the gelatinization of the contents of at least a portion of the internal cells of the juice sacs. Soon after the cell

contents gelatinize, or during the later stages of gelatinization, changes occur in the walls of these cells. Microchemical and histological studies have shown that these changes culminate in the formation of lignin or ligninlike substances. The walls of such cells (plate 3, *B*) become hard and woody, more or less like the wood cells in the trunk and limbs of the tree. As granulation progresses, these hard-walled cells and adjacent, thinner-walled cells disintegrate; an internal cavity is thereby formed in the juice sac (plate 4, *D*), and lateral pressures cause the sac to collapse (plate 4, *E*).

Gas Bubbles.—Gas bubbles appear within the cells at the same time that the walls are thickening and hardening. Examples are shown in

TABLE 20
CAROTIN CONCENTRATION IN "HEALTHY" AND GRANULATED
JUICE SACS
(Expressed in gammas per gram of fresh weight)

Year	"Healthy"* juice sacs	Granulated juice sacs	
		Stage 1	Stage 2
	<i>gammas</i>	<i>gammas</i>	<i>gammas</i>
1936.....	13.9	—	5.5
1937.....	18.3	10.0	4.7
Average†.....	16.1	10.0	5.1

* For explanation of "healthy," see text, p. 41.

† Gamma values of averages per mg. are: 0.0161, 0.0100, and 0.0051, respectively.

plate 4, *A*. Cells such as these may be easily isolated from the surrounding smaller cells, and the gas bubbles within them can be seen with the unaided eye. Lateral pressure on such a cell causes the gas bubble to shift position, but it cannot be forced out of the cell until the wall is ruptured. As the cell walls and the layers between them disintegrate, the gas passes out of the cells, forms gas pockets between them, and finally escapes into the large central cavity, the result of multiple cellular disintegration. Limited analytical tests on the composition of this gas showed, as would be expected, that it was composed largely of carbon dioxide.

Decrease in Color.—One of the early visible characteristics of granulation is the decrease in color (carotin) in the affected juice sacs. Spectroscopic and colorimetric determinations have shown that granulated juice sacs contain less and less carotin per gram of fresh weight as the disorder progresses. Table 20 shows the comparative amounts of carotin in "healthy" juice sacs and in those in the early and intermediate stages (stages 1 and 2) of granulation. "Healthy" and granulated sacs were taken from the same fruits. The results show that there was less than two thirds as much carotin per gram of fresh weight in the juice sacs in stage

1 of granulation as in those that were "healthy," and only one half as much in those in stage 2 as in those in stage 1. Tests were not made on juice sacs in stage 3 of granulation (collapsed sacs), but their visible appearance would lead to the conclusion that they contain only a trace of carotin, if any.

CAUSES OF GRANULATION

The cause or causes of granulation cannot be definitely stated. The production of granulation appears to be very closely related to those conditions which are responsible for vigorous tree growth and the production of large fruit sizes. In the majority of cases, granulation is much more prevalent in the fruit from young trees than in that from old trees. It may be abundant, however, in fruits produced on old trees that have been heavily pruned. Apparently the fruits from such trees are large and granulated because they have been borne on branches having luxuriant foliage like that on young trees. If this is true, then factors such as fertilizers, amount of irrigation water, rootstock, and climatic conditions may directly or indirectly influence the production of granulation. The fluctuation in the amount and severity of granulation from year to year indicates that climatic conditions may be an influencing factor.

The direct relation between granulation and large fruit sizes may be obscured by other factors, however. For example, the largest fruits are usually borne on the south side of the tree, but granulation is most abundant on the north side; one rootstock may cause the production of larger fruits but less granulation than another rootstock that produces slightly smaller fruits; and in the second year the fruits on girdled limbs may be smaller but contain more granulation than larger fruits on nongirdled limbs (table 10, p. 35).

Tree vigor and fruit size appear to have a definite influence on the amount and severity of granulation, yet the results of the experimental work show that other factors are also important. For example, in some cases a given tree, be it young or old, may constantly produce more granulation than an adjacent tree planted at the same time. This would indicate that some condition inherent in the tree itself may be even more important than cultural practices or soil and climatic conditions. The bud-selection studies as outlined in a previous section of this paper (see "Experiments in Budding and Top-Working," p. 16) throw some light on this phase of the problem. It should be remembered, however, that not all the trees studied showed the constant tendency to produce large or small amounts of granulation from year to year.

The fact that Valencias grown in the coastal areas usually have a lower content of total soluble solids than those grown in the interior

might be thought to be one reason why the former show more granulation than the latter (table 7, p. 25). During the years in which tests were made to determine the possible effect of the rootstock on the production of granulation, healthy fruits from trees grown on the same rootstocks near the coast and in the interior were analyzed for total soluble solids, acid, and for reducing and total sugars. The results of these analyses show that concentrations of all of these substances were lower, in every case, in the fruits from the coastal area (Tustin) than in those from the interior area (Riverside). Large fruits were found to have a lower concentration of these substances than smaller fruits, and, as a rule, to be more susceptible to granulation. The same holds true for the fruits from the inside and north side of the tree in comparison with those from other parts of the tree. Rough-lemon rootstock was one of the highest producers of granulation in both areas, and in almost every instance over a four-year period, fruits from these trees showed the lowest concentration of total soluble solids, acids, and sugars. Trifoliate orange, however, also one of the highest producers of granulation in both areas, was one of the highest producers of total soluble solids, acids, and sugars. Again, the Brazilian sour orange, which produced the highest average amount of granulation at Tustin and a fairly low amount at Riverside, was also among those which gave high analytical results at both places. Therefore, the analytical results do not entirely substantiate the suggestion that fruits having comparatively low concentrations of total soluble solids, acid, and sugars are most likely to granulate.

The suggestion has been made that the unequal distribution of soluble solids, primarily the sugars, in the mature orange fruit may be a factor in the production of granulation. If this were an important factor in granulation, then all mature Valencia oranges, both large and small, should be granulated, because they all have a higher concentration of soluble solids in the stylar end than in the stem end. Likewise, the mature navel orange and the grapefruit have a similar distribution of soluble solids, and yet, in California, these fruits are seldom if ever found to be granulated.

Granulation does not usually become visibly evident in the fruit until after the fruit has matured, and the soluble-solids content of the stem end of the fruit, where typical granulation begins, is then noticeably less than that of the stylar end. There are times, however, when a small mass of granulated sacs may be found near the center of the fruit, about a third or a fourth of the way in from the stylar end, where the soluble-solids content is highest, and not in any other portion of the fruit. In such cases, the granulated area is small, and the soluble-solids content

of the stylar end of the fruit remains higher than that of the stem end. Again, the marked difference in the soluble-solids content of the stem and stylar ends does not become evident until the fruit is approaching maturity (1), yet exceptional cases have been found where granulation was visible in large fruits at least two months before they were mature. Such evidence challenges the theory that the differences in soluble solids in the two ends of the fruit are an important factor in the production of granulation.

The possible relation of oil sprays to granulation was not experimentally determined in the series of tests reported in this paper, because groves were not available. The necessity for applying oil sprays to the groves year after year in the control of red mite made it impracticable to ask growers to offer their groves for such a study. However, in 1928 Higby *et al.*²⁴ applied twelve different brands or viscosities of oil sprays, at different times of the year, to twelve sets of 2 trees each in a grove in Orange County. With reference to granulation, the conclusions from these tests were: (1) that the time of application was not an important factor; (2) that the trees sprayed with heavy and medium oils produced much more granulation than those that were fumigated or than those sprayed with light oils; and (3) that the effects of oil sprays on the production of granulation in Orange County were much more marked than was generally reported for the interior districts. While the spray oils that are used today are much lighter than those used in 1928, many growers in the coastal area still feel that oil sprays are responsible for the production of at least a certain amount of granulation. It should be pointed out, however, that in the interior districts the oil sprays, as used today, appear to have little effect on the production of granulation. Recently experimental plots have been laid out in two widely separated groves, and the comparative effects of oil sprays and fumigation are being determined, but the data are not ready for publication.

The comparatively large number of influencing factors and the exceptions to the experimentally proved trends make it difficult to formulate any theory concerning the cause of typical granulation that will fit all cases.²⁵

²⁴ Higby, Ralph H., *et al.* A study of the effect of various oil sprays on the development and composition of Valencia oranges. Unpublished data, 2 (File 17):10-99, California Fruit Growers' Exchange Research Department, Ontario, California. 1928.

²⁵ After this paper went to press, an article by R. J. Benton appeared. Excerpts from this article follow.

In California, Dr. E. T. Bartholomew has been working on this problem for a number of years. He has not yet finished his studies, but as far as they have gone his work coincides with innumerable observations made in New South Wales. Dr. Bartholomew states that all rootstocks are prone to produce granulated fruit, but that it appears that less granulation occurs on trees irregularly watered than on trees generously irrigated. That is our opinion, as our view is that granulation results from the vegetative activity of the tree.

This condition can be almost regularly seen in our coastal districts on a large scale. . . . While all rootstocks will produce this effect [granulation], it does appear that the most vigorous stocks

CAUSES OF DRY JUICE SAC

Up to the present time, dry juice sac has always been classed by the citrus industry as a phase of granulation. But the condition of the juice sacs and the prevalence of the disorder in fruits of all sizes indicate that it has a different origin than granulation. No experimental work has been done to determine the cause of dry juice sac; therefore any suggestions in this line have to be based on observation and analogy.

In a previous article (4) the suggestion was made that dry juice sac (then classed as granulation) might be caused by temperatures that were not low enough to freeze the fruit and to effect the collapse of one or more segments, yet low enough to cause a physiological disturbance that would show in the fruit when it became overmature. This suggestion still appears to have merit, but it may be questioned, in view of the fact that dry juice sac was very abundant in the fruit during the latter part of the 1938 picking season, after the comparatively warm winter of 1937-38. That the fruits have lost much of their vitality and that the affected juice sacs are in a state of senility may be a more plausible suggestion. Past experience has shown that if the fruit is picked by the middle of July or by the first of August, very little trouble is experienced from this disorder. The affected juice sacs have largely lost their power to retain moisture, and as a result they shrivel, especially after the fruits have been removed from the tree.

CONTROL MEASURES

In the Field.—At the present time the only practicable way to avoid granulation is to pick the large fruits before they begin to granulate. Extra picks of this sort may have to be made from one to three or four times in a single season. The large fruits on the north and inside of the trees should receive special attention. Young groves and those growing on the lighter types of soil should be most closely watched, although many exceptions may be found. Careful observations should be made on individual trees and on certain areas within a given grove.

Lime spray has been found to reduce greatly the prevalence and severity of granulation in the fruits. But the amount of lime necessary to make the reduction commercially profitable is so large (at least 35 to 40 pounds per 100 gallons of water) that it apparently reduces the vitality

influence the earliest granulation. I believe the stock influence to be in the following order—rough lemon, Cleopatra mandarin and sweet orange, with trifoliata last. I feel confident that granulation is not a strain effect, for we have in several orchards valencias propagated from many sources. . . . All show granulation to about the same degree at the time of any examination late in the season.

. . . Heavy fertilising alone will not cause granulation, but in conjunction with ample irrigations it will encourage the development of this condition in late season oranges, if the trees are in a state to utilise such generous treatment. (Benton, R. J. Granulation. *Citrus News* 16(9): 129-30. 1940.)

of the tree. This conclusion is based on the fact that when the trees were subjected to the dry fall and winter winds, those which had been sprayed with lime in the spring lost many more leaves than those which had not been sprayed. It is possible that such a control measure might be used in localities where the dry winds are not such an important factor. Even in sheltered areas, however, the successive applications of lime spray in these amounts over a period of years might prove to be undesirable. So far as is known, no such tests have been made in California or elsewhere.

Limited tests have shown that the severity and amount of granulation may be noticeably diminished by reducing the amount of irrigation water applied. The reduction may cause at least a small decrease in yield, though not, apparently, in fruit size, and does not need to be very great in order to obtain a commercially important decrease in granulation. In the tests described in this paper, the decrease in yield was not apparent until the fifth year of treatment, while the decrease in amount of granulation was plainly evident after the first year. Although there was eventually a slight decrease in total yield, the yield of fancy and choice fruit was probably greater over the entire period because of the marked decrease in the amount and severity of granulation. The irrigation tests which appear to justify these statements were made, however, in only one locality (near Santa Aná) and on only one type of soil (Yolo loam), so that it is impossible to make a general recommendation for controlling granulation by this method.

It seems probable that any method of treatment which would prevent the production of large sizes would reduce the severity and amount of granulation in the fruit, because it is usually the thriftiest trees that produce the most granulation.

Present evidence indicates rather strongly that the control or lessening of granulation in the future depends upon careful choice of root-stock and that for the starting of trees for new groves and for replanting, buds should be taken from trees that have shown a minimum of granulation during the early years of their growth.

In the Packing-House.—Evidence to date appears to indicate that typical granulation does not become materially worse after the fruit has been brought to the packing-house. It is true that some of the juice sacs which appeared to be healthy, or nearly so, when the fruit was picked will lose moisture and tend to shrink. For this reason the disorder may appear to spread in the tissues during storage or transit, but after the fruit has been removed from the tree, apparently no new juice sacs become affected, nor do those already affected continue to enlarge and harden. Granulation of the juice sacs is a growth phenomenon; therefore it is scarcely more probable that healthy juice sacs should become

granulated after the fruit has been picked than that the fruit itself should grow after it has been removed from the tree.

Since, as a rule, the disorder cannot be reliably detected by any surface markings, samples have to be cut and different portions of a given lot of fruit discarded or graded accordingly. The fluoroscope as used in the packing-houses at the present time is useful in detecting the severely granulated fruits, but is of little aid when the fruits are only slightly or moderately granulated.

The type of trouble which is usually referred to as "blossom-end (stylar-end) granulation," has been described in an earlier portion of this paper (see "Description of Granulation and Dry Juice Sac," p. 4), and "dry juice sac" has been suggested as a suitable name for it. This disorder becomes worse as the fruit is held in the packing-house and can be detected only by cutting the fruit or, in the moderate or severe stages, by the use of the fluoroscope. Little can be done to prevent its spread in the fruit, because the real damage was done before the fruit was picked. Close watch should be kept on all lots of fruit that are to be shipped after the middle of July. The fruit may appear to be reasonably sound when picked. Since the affected sacs have largely lost their power to retain moisture, storage at a comparatively high humidity is the only suggestion that can be made for temporarily retarding the spread of the disorder within the fruit. Such fruit should be consumed as soon as possible and should not be sent to eastern markets.

SUMMARY

Granulation has been reported and accurately described from nine foreign countries, and it is probably present in one or more varieties wherever citrus is grown.

The Valencia orange is the only citrus variety in California in which granulation is commercially important.

In California, granulation is more prevalent in the coastal than in the interior districts.

The fruit on young trees is more subject to granulation than that on old trees.

Granulation in California does not usually become commercially important until after the middle of the picking season.

Reduction of the amount of light by shading trees did not cause an increase in the amount of granulation in the fruit.

Granulation originates in and is usually confined to the stem half of the fruit pulp.

In spite of the fact that juice sacs usually become enlarged as they become granulated, there is not a consistent enlargement of the stem end

of granulated fruits; in most cases the effect of the enlargement appears to be distributed over the entire fruit.

Spraying the trees with lime apparently reduced the amount of granulation in proportion to the concentration of lime used. Lime spray is not recommended as a control, however, because of the effect on the trees during desiccating winds.

There is a strong tendency for certain trees in a given grove to be consistent from year to year in the production of much or little granulation.

Buds from high and low granulation-producing trees are being grown on both sweet- and sour-orange stocks to determine whether the tendencies toward the production of granulation may be transmitted through the bud. Other trees have been cross-top-worked for the same reason.

Trees affected with scaly bark do not, as a rule, produce a higher percentage of granulation than those not affected.

Reduction in frequency of application and in amounts of irrigation water resulted in a slight reduction of fruit yield in the last (fifth) year of the treatment, but not in fruit sizes. After the first year of differential treatment, the trees on the wet plots produced much more granulation than those on the dry plots. Analysis showed that there were no appreciable differences in total soluble solids, acids, pH, and sugars in fruit samples from the wet and from the dry irrigation plots.

Certain rootstocks may augment the amounts of granulation produced, but the tendencies in this direction may not be the same in different localities.

Low temperatures may augment granulation in fruits that are inclined to granulate anyway, but apparently not in others.

Limb girdling did not increase fruit size or have any effect on amount of granulation in the season after girdling. In the second year, there was a general decrease in the size of fruits on the girdled limbs, but there was more granulation in these fruits than in the larger fruits from the limbs that were not girdled.

In general, the larger the fruit, the more likely it is to be granulated.

Granulation is more prevalent in fruits from the inside and from the north side of the tree than in fruits from any other portion of the tree.

The severity of granulation may range all the way from granulation of only two or three juice sacs to granulation of practically the entire pulp of the fruit.

There appears to be no difference in the nitrogen content of healthy and granulated juice sacs.

Large fruits have a lower total soluble-solids content than small ones, but small fruits are sometimes as severely granulated as large ones.

Granulated juice sacs have a higher percentage concentration of moisture and inorganic matter and more dry matter per juice sac, but a lower active and total acidity and lower concentration of sugars than healthy juice sacs.

Juice sacs in stage 2 of granulation contain approximately twice as much total pectin as those that are in a healthy condition.

Juice sacs usually enlarge and become comparatively hard during the earlier stages of granulation and then soften and collapse during the later stages. The enlargement is due to cell enlargement and not to cell multiplication. The hardening is due to gelatinization of the cell contents and lignification of the walls of some of the internal cells. The softening and collapse are caused by the progressive disintegration of many of the internal cells. Occasionally juice sacs may become granulated without becoming very much enlarged.

In the intermediate stages of granulation, gas forms and is temporarily trapped within the hard-walled cells. Later the gas escapes into newly formed intercellular spaces and, finally, into the cavity formed by cellular disintegration.

The concentration of coloring matter (carotin) in the juice sacs decreases as granulation progresses.

The actual cause of granulation has not been definitely determined, but some of the possible causes are discussed.

Control measures are limited, but various phases of the problem are discussed.

The apparent inconsistencies in some of the results and the exceptions to experimentally proved trends indicate that a comparatively large number of factors are concerned in the production of granulation.

Extensive laboratory studies have shown that many changes occur in the juice sacs during the process of granulation. As a result of these changes and, no doubt, others that have not been mentioned, it is not surprising that the taste and edibility of the affected fruits have been greatly modified.

The preceding summary statements refer to typical granulation and not to "dry juice sac." The latter has been suggested in this paper for the first time as a suitable descriptive term for the physiological disorder which in the past has been called "blossom-end granulation."

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